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.**

RESUMEN. Con el objetivo de caracterizar la glándula adrenal humana en esta
do fetal (10 a 12 semanas) y adulta (post-morten) se determinaron las actividades específicas de las 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 detec-
ción 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 lige-
ro 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 significa-
tivamente mayor (p < 0,001) en el tejido fetal. La actividad de la GST fue tambiñ
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 xenobióticos y toxinas
endógenas. Estos resultados pudieran contribuir a la explicación de los resul-
tados del implante de células catecolaminérgicas en la enfermedad de
Parkinson.

INTRODUCTION

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 adrena-
line (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 electro-
chemical 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). GSTsa 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 GSTsa between both adrenal
medulla may reflect a different capacity of these tissues to metabolize xenobióticos
and endogenous toxins. This could explain the performance of catecholaminergic
cells in human PD implants.
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) and dopamine (DA) in both HFAG and HAAG, using a HPLC procedure with electrochemical detection. The adrenal tissue was homogenized (1:5 weight:volume) in glass potters using 0.1 mol/L HClO₄ containing NaHSO₃ (1.9 mol/mL) as an antioxidant.

Chromaffin cells have been widely explored as a potential source of CA in animal models and human cases of striatal dopamine deficiency. In general, the recovery of motor function following implantation has been quite modest, which may be related to the poor survival rate of implanted chromaffin cells. 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 is a vast number of enzyme systems such as monoamine oxidase, superoxide dismutase and glutathione-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). The purpose of the present study was to characterize both adult and fetal adrenal glands and fetal (10-12 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. This allowed the easy obtaining of the tissue in the age range studied.

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.

Tissue sampling

Human fetal adrenal glands (HFAG) were obtained (4.0 ± 2.1) h post mortem, (mean ± SD) from six male patients (2.4 ± 3.4) years of age (means ± SD) with no known history of neuropsychiatry or neurodegenerative disorders. (Table 1).

Table 1. Fetuses and adults employed according to age.

<table>
<thead>
<tr>
<th>N</th>
<th>Age</th>
</tr>
</thead>
<tbody>
<tr>
<td>5</td>
<td>10 weeks</td>
</tr>
<tr>
<td>5</td>
<td>11 weeks</td>
</tr>
<tr>
<td>5</td>
<td>12 weeks</td>
</tr>
<tr>
<td>6</td>
<td>(62 ± 5.6) years</td>
</tr>
</tbody>
</table>

The highest relative amount of DA was detected in the 10-12 week old fetuses, (8.31 % of the total CA), 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. Protein concentration was measured by the Bradford method, 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.

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 the 10-12 week old fetuses, (8.31 % of the total CA), glutathione-S-transferase specific activity (GSTsa)

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Fetal and adult adrenal gland. TH activity was assessed in adult and fetal adrenomedulla homogenates. TH activity was significantly greater in the adult tissue (Table 3).

There were differences in THSₐ at different fetal ages. THSₐ was significantly higher in 11 to 12 week fetuses (p < 0.01) when compared with the 10-week-old fetuses. In general, THSₐ 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 (GSTSₐ) was assessed in adult and fetal adrenomedulla homogenates (Table 3).

GSTSₐ was shown to be significantly higher (p < 0.001) in the adult group. Unlike the results for THSₐ, GSTSₐ was not seen to vary significantly over the fetal ages studied.

**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 obtain (Table 1).

Fetal CA is produced primarily in the adrenomedulla. 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 Zuckerkandl.

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 a accompanied by a slight increase in the levels of A. This is in agreement with previously published results.

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 THSₐ 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).

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

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

**Table 3.** Tyrosine hydroxylase specific activity (THSₐ) and glutathione-S-transferase specific activity (GSTSₐ) in fetal and adult human adrenomedulla.

<table>
<thead>
<tr>
<th>Source</th>
<th>Age</th>
<th>N</th>
<th>THSₐ</th>
<th>GSTSₐ</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fetus</td>
<td>10 weeks</td>
<td>5</td>
<td>2.61 ± 0.37*</td>
<td>0.0281 ± 0.0011</td>
</tr>
<tr>
<td>Fetus</td>
<td>11 weeks</td>
<td>5</td>
<td>9.95 ± 1.76</td>
<td>0.0303 ± 0.0016</td>
</tr>
<tr>
<td>Fetus</td>
<td>12 weeks</td>
<td>5</td>
<td>11.36 ± 0.70</td>
<td>0.0290 ± 0.0012</td>
</tr>
<tr>
<td>Adult</td>
<td>(62.4 ± 3.4) years</td>
<td>6</td>
<td>39.66 ± 3.76</td>
<td>0.3134 ± 0.0151*</td>
</tr>
</tbody>
</table>

THSₐ is expressed in pmol L-DOPA • min⁻¹ • mg of protein⁻¹. GSTSₐ 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.

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.

The GST are believed to play an important role in the protection of cellular macromolecules from attack by reactive electrophiles. GST functions as an intracellular detoxication 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 GSTSₐ 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.

<table>
<thead>
<tr>
<th>Age</th>
<th>N</th>
<th>NA</th>
<th>A</th>
<th>DA</th>
</tr>
</thead>
<tbody>
<tr>
<td>10 weeks</td>
<td>5</td>
<td>27.18 ± 3.01</td>
<td>3.06 ± 1.46*</td>
<td>0.56 ± 0.14</td>
</tr>
<tr>
<td>11 weeks</td>
<td>5</td>
<td>20.8 ± 2.16</td>
<td>5.41 ± 0.36</td>
<td>0.40 ± 0.10</td>
</tr>
<tr>
<td>12 weeks</td>
<td>4</td>
<td>15.06 ± 1.06*</td>
<td>8.01 ± 0.03</td>
<td>2.09 ± 0.06*</td>
</tr>
<tr>
<td>62 years</td>
<td>6</td>
<td>58.2 ± 11.26</td>
<td>352.3 ± 114.9</td>
<td>0.19 ± 0.07*</td>
</tr>
</tbody>
</table>

The values are the means ± SD, expressed in nanogram/milligram of wet weight. *p < 0.01 for differences from 10 week to 11 or 12 weeks. *p < 0.001 for differences 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 increased 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 endogenous toxins.

Finally it is well known that GST M2-2 catalyze the detoxification of catecholamines acting as neuroprotective antioxidant system. On the other hand recently was demonstrated that GST pi was a dopaminergic neuron induced suppressor of dopamine induced apoptosis in PC12 cells through inhibition of Jun-N-terminal Kinase (JNK). These well-documented results are example of the relationship between catecholamine pathway and detoxifying enzymes like GST. It is also demonstrated 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.

**BIBLIOGRAPHY**