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Short Communication

High-performance liquid chromatographic determination of norepinephrine, epinephrine and dopamine in human foetal adrenal gland

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Abstract

The purpose of this work was to characterize the human foetal adrenal gland (HFAG) by studying norepinephrine (NE), epinephrine (E), and dopamine (DA) levels in foetuses with ages ranging from 10 to 18 weeks, as a source of chromaffin cells for basic and clinical research, in the treatment of Parkinson's disease. The HFAGs were obtained from ten abortions with foetal ages ranging from 10–12 ($n = 7$) to 13–18 ($n = 3$) weeks. For the simultaneous detection of NE, E, and DA a high-performance liquid chromatographic (HPLC) procedure with electrochemical detection was employed. The concentrations found (ng/mg wet weight) were 24.24 ± 7.06 for NE, 3.56 ± 2.97 for E and 0.45 ± 0.27 for DA at 10–12 weeks and 14.91 ± 9.94 for NE, 8.18 ± 8.79 for E and 0.16 ± 0.05 for DA at 13–18 weeks. The DA:E ratio present in HFAG between 10 and 12 weeks was 100 times higher than that reported by other authors in adult adrenal medulla.

1. Introduction

Motor defects characteristic of Parkinson's disease (PD) are attributed to a decrease of dopaminergic innervation in the striatum [1,2]. That is why cell transplantation in animal models has been carried out employing tissues capable to restore, at least partially, the nigrostriatal dopaminergic pathway using foetal substantia nigra (SN) [3] or tissues that act as a biological source of dopamine (DA), such as the suprarenal medulla. The DA present in the adult adrenal medulla has been interpreted as an intermediary metabolite in the biosynthesis of epinephrine (E) [4,5] (Fig. 1).

Studies using human adult adrenal medulla have been frequently reported [6,7]. However, the human foetal adrenal gland (HFAG) has been scarcely studied, in spite of its greater functional plasticity [8,9] and the numerous reports showing the advantages of using young or foetal donor tissues [10,11].

The purpose of the current study was to

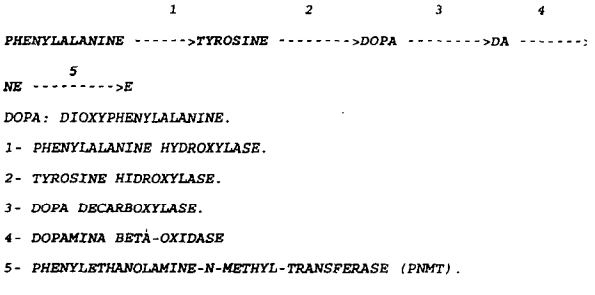

Fig. 1. Catecholamine pathway.

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characterize HFAG by studying NE, E and DA levels in foetuses with ages ranging from 10 to 18 weeks.

2. Experimental

2.1. Tissue sampling

Human foetal adrenal gland (HFAG) was obtained with the aid of a stereomicroscope from foetuses of therapeutical abortions in the Eusebio Hernández Maternal Hospital. HFAG can be clearly distinguished at a foetal age of six weeks [12]. This allowed the easy obtainment of the tissue in the age range studied (Table 1). The glands were stored at −70°C until catecholamines were assayed.

2.2. Catecholamine assay

For the simultaneous detection of NE, E and DA in HFAG an HPLC procedure with electrochemical detection was employed. The adrenal tissue was homogenized (1:5 wet weight:volume) in glass potters using 0.1 *M* $HClO_4$ containing $NaHSO_3$ (1.9 m*M*) as an antioxidant. The homogenate was immediately centrifuged for 30 min at 10 000 *g* at 4°C and the protein free supernatants were passed through a 0.22-μm membrane filter. Dihydroxybenzylamine was used as internal standard. A sample volume of 5–20 μl was injected onto a Unicam isocratic HPLC System consisting of a PU 4100 pump, and a PU 4022 electrochemical detector set at 70 mV and 30 nA of sensitivity.

Table 1
Foetuses employed according to age

N	Weeks
3	10
1	10–11
2	11
1	11–12
1	13
1	14
1	18

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

3. Results and discussion

Foetal catecholamines are synthesized mainly in the adrenal medulla from the amino acids phenylalanine and tyrosine, as shown in Fig. 1.

Figs. 2 and 3 show chromatograms of a mixture of NE, E, and DA standards and of a HFAG sample, respectively.

The data in Table 2 show that the main catecholamine for all the foetal ages studied was

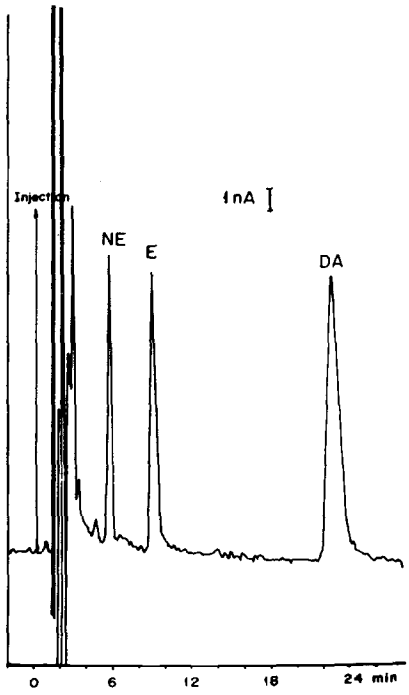

Fig. 2. Chromatogram of NE, E, and DA standards. A volume of 25 μl containing 500 pg of NE, E and 1000 pg of DA was injected.

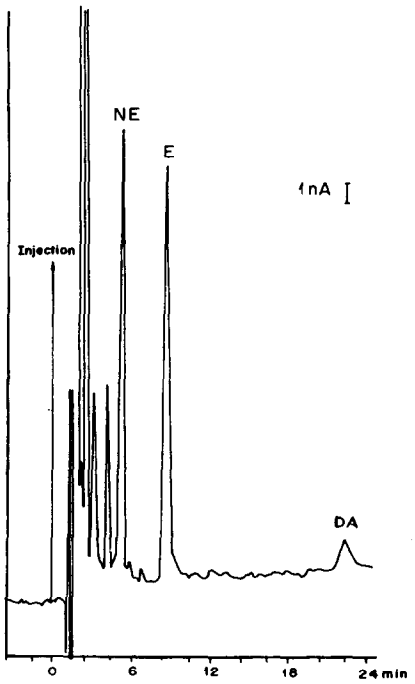

Fig. 3. Chromatogram of human foetal adrenal gland sample (age 10 weeks). Peaks corresponding to NE, E and DA are indicated.

NE. A decrease in the level of NE with increasing foetal age was observed. This decrease is accompanied by a slight increase in the levels of E, although at the ages of 13, 14 and 18 weeks considerable variation in the amounts of catecholamines was found. This is in accordance with previously published results [13].

NE is the main catecholamine in prenatal life. The amount of NE relative to the total amount of catecholamines begins to decrease only after birth, when a significant increase in the amount of E occurs [14]. In foetuses of 10–12 weeks old the highest relative amount of DA was detected, representing 1.58% of the total catecholamines (Table 2). The DA:E and DA:NE ratios found in the HFAG between 10 and 12 weeks were 1:8 and 1:54 respectively, *i.e.* 100 and 1.3 times higher than the ratios reported for human adult adrenal gland [15]. The striking difference in the DA:E ratio may be explained by increased synthesis of DA (relative to the total catecholamines synthesis) or by a delay of its degradation in the foetal age range studied, compared with the adult. Moreover, a decrease in the relative amount of E in the HFAG, cannot be excluded. The DA:E ratio observed here is similar to that reported for HFAG of 15 to 18 weeks old [16]. The relative amounts of DA are 10 times higher than those reported for adult medulla [15].

On the other hand, it is known that the chromaffin cells are targets for different kinds of neurotrophic factors [17], and that they respond to the nerve growth factor (NGF) with selective induction of the enzymes tyrosine hydroxylase (TH) and dopamine β-hydroxylase (DBH) [18,19]. Variations in the kind and concentration of these neurotrophic factors between foetal and adult tissue might explain the relative increase of DA in foetal tissue.

NE is the major catecholamine in the foetal period. This may be explained by the fact that the enzyme phenylethanolamine-N-methyl-transferase (PNMT), which is responsible for the synthesis of E from NE, is mainly found in the

Table 2
Levels of NE, E and DA in human foetal adrenal gland

Age (weeks)	*n*	Concentration (mean ± S.D.) (ng/mg wet weight)		
		NE	E	DA
10–12	7	24.43 ± 7.06 (85.90)	3.56 ± 2.97 (12.51)	0.45 ± 0.27 (1.58)
13–18	3	14.91 ± 7.94 (54.3)	8.18 ± 8.79 (35.18)	0.16 ± 0.05 (0.69)

Numbers in brackets are the relative percentages of the total catecholamines detected.

medulla, which is immature at this embryonic stage (10–12 weeks); the activity of PNMT increases with foetal age. PNMT is modulated by glucocorticoids [20,21] that are present in the cortex, which is also still immature at this age. Thus, there is only a partial modulation. This could explain the relatively low levels of E observed in foetal tissue, where we detected levels 6.3 times lower than those reported in the adult medulla [15]. The relative increase in DA in foetal gland compared to the level of this catecholamine in the adult medulla could indicate that the foetal adrenal tissue is an alternative source of dopaminergic cells in grafts. Supporting this idea, Kamo *et al.* [22] reported improvement of the rotational behaviour induced in rats lesioned with 6-hydroxy-dopamine, after grafting HFAG tissue.

4. Conclusions

The results of this study lead to the following conclusions:

(1) NE is the main catecholamine in the adrenal foetal glands of the foetal ages studies. (2) The DA:E ratio found in HFAG between 10 and 12 weeks is 100 times higher than that reported by other authors in adult adrenal medulla. (3) Our results support the idea of using HFAG as an alternative dopaminergic source for experimental grafting.

5. References

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