Distribution of Noradrenaline and of Monoamine Oxidase and Catechol-*O*-Methyltransferase Activity in Human Foetal Heart

The content of noradrenaline in the adult heart has been studied in several mammalian species[1-5]. The atria generally have a higher noradrenaline concentration than the ventricles, which is in accord with the observation that the adrenergic nerve supply is most abundantly found in the atrial myocardium[6]. In the present investigation the distribution of noradrenaline in the different parts of the heart was studied in the human foetus and compared with the activity of the noradrenaline degrading enzymes, i.e. monoamine oxidase and catechol-*O*-methyltransferase. The enzyme activity was also measured in the tissue adjacent to the heart base between the ascending aorta and the pulmonary trunk (= inferior aorticopulmonary space).

Material and methods. Hearts were obtained from 32 human foetuses at therapeutic abortions performed by laparotomy or curettage. The gestational (menstrual) age of the foetuses ranged from 9 to 24 weeks. The hearts were freed from adjacent organs within 5 min of the removal of the foetus from the uterus and placed on an ice-cooled Petri dish. The heart specimens were blotted on filter paper and weighed. 11 of the hearts (foetal crown-heel length 15–31 cm) (group A), which were used for determination of noradrenaline, were dissected into 5 parts: right atrium, left atrium, right ventricle, left ventricle and intraventricular septum. These parts were quickly cut into smaller fragments and homogenized in ice-cooled perchloric acid. The content of noradrenaline was measured spectrofluorimetrically according to BERTLER et al.[7], as modified by HÄGGENDAL[8]. The noradrenaline of the heart was identified by comparing the excitation and emission spectra with that of authentic noradrenaline.

Hearts from another 12 foetuses (group B) obtained at an earlier stage of gestation (9th–11th week) were divided in a plane between the atria and the ventricles. The atrial parts (which also contained the most proximal portions of the ascending aorta and the pulmonary trunk) and the ventricular parts were pooled, and their noradrenaline content was determined in the same way as in group I.

9 hearts (foetal crown-heel length 16–25 cm) intended for enzyme assay (group C) were divided in the same way as in group I, weighed and frozen immediately in liquid nitrogen. In 6 of these cases tissue specimens from the inferior aortico-pulmonary space were included. The specimens were stored at −70 °C for up to 16 days before determination of MAO and COMT activity. Control analyses showed no decrease of the enzyme activities within this period.

The tissue was homogenized in 40 volumes (w/v) of ice-cold isotonic KCl solution. After centrifugation ($8000 \times g$, 15 min) the supernatant served as enzyme

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Table I. Distribution of noradrenaline (μg/g wet weight) in human foetal hearts

Foetal crown-heel length (cm)	Right atrium	Right ventricle	Left atrium	Left ventricle	Interventricular septum
15	0.30	0.26	0.50	0.22	0.34
19	0.18	0.25	0.42	0.25	0.26
20	0.45	0.34	0.46	0.24	0.32
20	0.25	0.26	0.34	0.19	0.22
20	0.31	0.60	0.48	0.53	0.70
20	0.19	0.18	0.19	0.18	0.21
21	0.28	0.24	0.32	0.25	0.32
22	0.28	0.31	0.46	0.37	0.32
25	0.31	0.32	0.37	0.30	0.38
25	0.28	0.34	0.33	0.31	0.29
31	0.27	0.28	0.32	0.31	0.32
Mean ± S.E.M.	0.28 ± 0.022	0.31 ± 0.034	0.38 ± 0.086	0.29 ± 0.029	0.33 ± 0.042

Table II. Distribution of noradrenaline (total weight and concentration) in young human foetal hearts

No. of hearts	Foetal age	Noradrenaline			
		Atria + mediastinum		Ventricles	
		Content (μg)	Concentration (μg/g)	Content (μg)	Concentrytion (μg/g)
12	⩽ 12 weeks	0.22	0.24	0.03	0.05

Table III. Monoamineoxidase activity in human foetal hearts (nmol formed product per mg protein per 60 min incubation)

Foetal crown-heel length (cm)	Right atrium	Left atrium	Right ventricle	Left ventricle	Intraventricular septum	Inf. aortico-pulmonary space
16	2.33	1.48	1.82	0.97	1.39	1.90
18	0.88	1.36	1.48	0.93	2.09	0.76
19	0.28	0.35	1.20	0.61	1.44	–
20	0.73	1.63	2.43	1.75	1.86	2.92
20	1.03	2.00	1.50	1.04	1.10	2.54
21	0.77	0.65	1.48	0.58	1.46	–
22	1.80	1.91	1.95	1.83	1.86	3.50
23	1.28	1.81	3.26	1.96	2.00	2.68
25	0.53	0.45	2.83	2.29	2.72	–
Mean ± S.E.M.	1.07 ± 0.21	1.29 ± 0.21	1.99 ± 0.23	1.33 ± 0.21	1.77 ± 0.16	2.38 ± 0.39

Table IV. COMT activity in human foetal hearts (pmol formed product per mg protein per 60 min incubation)

Foetal crown-heel length (cm)	Right atrium	Left atrium	Right ventricle	Left ventricle	Intraventricular septum
16	3.6	5.1	5.0	0.0	0.0
18	2.2	6.7	11.0	6.7	9.2
19	0.0	6.1	6.2	5.9	13.2
20	0.0	5.7	18.9	15.7	8.4
20	0.7	5.6	12.0	5.6	6.2
21	0.0	12.1	5.9	12.1	14.4
22	0.0	20.5	11.3	20.0	18.4
23	1.0	0.0	11.5	0.0	15.9
25	0.0	7.8	5.6	7.9	2.7
Mean ± S.E.M.	0.83 ± 0.43	7.73 ± 1.90	9.71 ± 1.50	8.21 ± 2.23	9.82 ± 2.06

solution. The MAO activity (oxidation of kynuramine to 4-hydroxyquinoline) of the various tissues was measured by the fluorimetric method described by KRAJL[9]. COMT activity was measured by a modification of a method described by AXELROD et al.[10]. Instead of radioactive substrate we used the isotope labelled cofactor S-adenosyl methionine – C^3H_3 (NEN-Chemicals). The substrate was adrenaline and the formed radioactive metaadrenaline was extracted from the incubation mixture with isomylalcoholtoluol and after addition of Bray's solution measured in a scintillation counter (Packard). Both MAO and COMT activities were expressed as μmoles formed product per mg protein per 60 min incubation.

The protein content of the heart tissue was determined by the method of LOWRY et al.[11]. Duplicate tests were performed.

During the operation the mothers received thiopenthal sodium, nitrous oxide, and (in group A and C) fluothane and suxamethonium chloride. Statistical analyses were performed with Student's *t*-test.

Results. The concentration of noradrenaline in the hearts of group A and C are summarized in Table I and II. In the younger foetus (group B) the noradrenaline concentration of the ventricles was markedly lower than that of the cranial parts of the hearts, and also lower than that of the ventricles of the older hearts (group A). The concentration of the catecholamine was evenly distributed among the different chamber walls of the older foetus group. There was thus no significant difference between the atrial and the ventricular part, nor between left and right half of the older hearts.

The distribution of MAO activity in the foetal hearts is given in Table III. High MAO activity was found in the right ventricle and in the muscular interventricular septum. The lowest MAO activity was obtained in the right atrium. The MAO activity in the left ventricle increased with foetal length ($r = 0.7256$). In the inferior aortico-pulmonary space the MAO activity exceeded that found anywhere in the hearts and showed a positive correlation with foetal length ($r = 0.6818$).

The activity of COMT was distributed uniformly among the various parts of the hearts except in the right atrium where it was very low (see Table IV). The COMT activity in the heart was, on the average, one tenth to one hundredth that of the MAO activity in the corresponding parts of the heart. No COMT activity was found in the lower aortico-pulmonary area.

Discussion. The distribution of the noradrenaline concentration in the youngest human foetal hearts suggests that the synthesis and/or the storage of noradrenaline starts at the base of the heart during the ontogenesis. The very low concentration of noradrenaline in the ventricles at this developmental stage is in agreement with the findings that before the 11th–12th week of gestation only few or no noradrenaline containing nerve fibres are

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visible with the fluorescence method by FALCK and HILLARP in human heart ventricles (GENNSER and OWMAN, to be published).

In the older foetus (group A) the concentration of cardiac noradrenaline in every heart chamber was lower than that reported for adult sub-primate mammals. It was apparent that the pattern of noradrenaline distribution between atria and ventricles seen in adults of other species[1-5] is not attained at this stage of human development.

The activities of MAO and COMT were of approximately uniform magnitude in the different parts of the heart with 2 notable exceptions: the MAO activity of the right ventricle exceeded that of the left ventricle ($p < 0.001$) and the right atrium contained a very low COMT activity compared with the other heart chambers. It was a very conspicuous finding that the major enzyme responsible for the degradation of circulating noradrenaline had a low activity in a region containing the cardiac pacemaker. In the absence of neuronal uptake, inhibition of COMT may cause a significant increase of noradrenaline concentration at the adrenergic receptor sites if the concentration of exogenous noradrenaline is low[12]. It seems reasonable to assume that the neuronal uptake mechanism of noradrenaline (uptake U_2) is relatively ineffective in the sparsely innervated foetal heart during the early half of ontogenesis. The very low COMT activity in the right atrium therefore appears to be of special significance. A local reduction of the ability to metabolize noradrenaline in the sinus region, as suggested by the low degrading enzyme activity in vitro, might be of importance for the adrenergic regulation of the foetal heart rate, before the nerve-receptor function in the sinus node is fully established. A positive chronotropic effect exerted by adrenaline was recently demonstrated already in the very young (and not yet innervated) human foetal heart[13]. Catecholamines (noradrenaline and adrenaline) are present in the human foetal adrenals[14,15] and extra-adrenal chromaffin tissue[14,16] at an early stage. Such non-neuronal amines would reach the receptors of the sinus node by the circulation in far lower concentrations than that of the transmitter released from adrenergic nerve terminals close to the neuromuscular junction. Thus, if non-neuronal adrenergic regulation of the foetal heart rate via the sinus node is present at this developmental stage, the activity of extraneuronal enzymatic inactivation by COMT must apparently be very low or non-existing.

The tissue specimen taken between the ascending aorta and the pulmonary trunk includes the inferior aorticopulmonary glomus. This cluster of argyrophil and probably also chromaffin cells in the human foetus has been attributed a possible chemoreceptor function[17]. The high MAO activity of this region demonstrated in the present study is of interest in view of the assumption that catecholamines are involved in this mechanism[17,18].

Zusammenfassung. Die Konzentration von Noradrenalin und die Aktivität der MAO und COMT wurde in verschiedenen Teilen des menschlichen foetalen Herzens bestimmt. Die Ventrikel der Herzen (bis zu 12 Wochen alt) waren sehr noradrenalinarm. Die COMT-Aktivität des rechten Vorhofs war bis zur Mitte der Schwangerschaft viel niedriger als die der übrigen Herzgebiete. Die COMT- und MAO-Aktivität im Gewebe des Spatium zwischen der Aorta und der Arteria pulmonalis lag ungefähr in der gleichen Grössenordnung wie die der Ventrikel.

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Normalization by Nucleotides of Impairments in Deamination of Nitrogenous Compounds in Liver of Tumor-Bearing Mice

Monoamine oxidase activity was decreased[1] in liver of mice with ascites carcinoma. In tissues of the tumor-bearing animals lipid peroxides[2] accumulated, which resemble oxidized oleic acid. Treatment with oxidized oleic acid[3,4] or with ergosterol peroxide[5] of highly purified monoamine oxidases inhibited deamination of monoamines and, at the same time, caused reversible transformation in catalytic properties of the enzymes: the latter acquired abilities to deaminate diamines, polyamines, AMP, lysine and other nitrogenous compounds. If a similar transformation occurred in tumor-bearing mice we could expect that the decrease in monoamine oxidase activity in their liver will be accompanied by appearance of (or increase in) deamination of putrescine or AMP.

Cells of Ehrlich ascites carcinoma were obtained at the 7th day of development of the tumor and inoculated i.p. (about 10^7 cells per each animal) to white (10–20 g) mice. Isolation of mitochondria and estimation of the deamination rates were performed as described previously[6].

In liver mitochondria of the tumor-bearing mice deamination of tyramine or serotonin was decreased, deamination of AMP was sharply increased and an ability to de-

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