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Studies of Auricular Catecholamines by Fluorescence Histochemistry in Various Heart Diseases of Man

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Summary. A comparative histochemical and clinical study concerning the state of the intrinsic adrenergic innervation of the human atrial myocardium was carried out, using the glyoxylic acid-induced fluorescence histochemical method. Specimens from the right auricular appendage were obtained during open-heart surgery from patients suffering from 1. ischaemic heart disease (IHD), 2. atrial septal defect of the secundum type (ASD), and 3. left-sided univalvular or multivalvular heart disease (VHD) with or without congestive heart failure (CHF) experienced prior to surgery. In the IHD group the densities of both the perivascular and the "free" myocardial adrenergic nerve net were greater than in the ASD group and especially in the VHD/CHF group. Secondly, the intensity of fluorescence of the adrenergic structures was generally higher in the IHD group than that in the VHD/CHF group. Further, the average size of the varicosities, the number of varicosities per given length of axon, and the proportional share of the large varicosities were greater in the IHD group than in the ASD and VHD/CHF groups. The difference between the IHD and ASD groups was not great but was obvious in any case. In some patients with VHD/CHF fluorescing axons were observed only occasionally, and the tiny varicosities exhibited a hardly discernible fluorescence. Thus the amount of noradrenaline (NA) in the adrenergic fibres in the IHD group seems to be higher than in the ASD and especially VHD/CHF groups. The high level of NA in the IHD group is assumed to constitute a contributory factor in both intracellular metabolic changes and the systemic changes typical of myocardial ischaemia and infarction. In one patient with IHD and in six patients with VHD/CHF with significantly higher heart volume (mean $\pm$ SD) compared with the rest of

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the patients ($P < 0.001$), huge local axonal accumulations of NA in the form of "droplet fibres" were found. These enlarged, bulging adrenergic axons are assumed to be a consequence of mechanical trauma with stretching or disruption of the axons due to myodegenerative processes. It is further assumed that these "droplet fibres" are relatively common in those patients with diseased myocardium. They may constitute an extra contributory factor to the tendency to arrhythmias so typical of patients of this kind, by increasing the excitability of non-automatic tissue.

Key words: Heart diseases – Adrenergic innervation – Fluorescence histochemistry – Catecholamines.

Introduction

The content of noradrenaline (NA) in human heart is highest in the auricles and lowest in the left ventricle (Chidsey et al., 1965). Earlier a positive correlation has been shown to exist between auricular and ventricular NA contents (Chidsey et al., 1965). In the failing heart there is a reduction of myocardial NA stores (Chidsey et al., 1963). The decline is a secondary phenomenon derived from an abnormality of synthesis and neuronal binding of NA (Spann et al., 1965; Braunwald, 1975). In contrast to this reduction of NA in myocardial failure in ischaemic heart disease (IHD) the NA content in right auricular tissue is rather high (Penttilä et al., 1975, 1977). The depletion of NA in myocardial failure can be shown in animals (Vogel et al., 1969) by the conventional formaldehyde-induced fluorescence (FIF) histochemical method, which, is however not suitable for studies on humans because of the strong autofluorescence of connective tissue, typical of primates (Baumgarten, 1967). This technical difficulty can be avoided by using the glyoxylic acid-induced fluorescence (GIF) histochemical method introduced recently (Lindvall and Björklund, 1974), which has been shown to be very useful for studies on humans (Kyösola et al., 1976).

Catecholamines (CA) have a central role both in physiological and pathophysiological situations in heart function. The aim of the present investigation is to compare the adrenergic network in samples of right atrial appendage of groups of patients suffering from various heart diseases by using the GIF histochemical method.

Patients and Methods

Classification of the Patients

Samples of the right atrial appendage were obtained during coronary bypass surgery from seven patients, one female and six males, suffering from IHD. Their age varied from 31 to 54 years. They were selected for the operation according to anamnestic, clinical and coronary angiographic data. The youngest patient (patient no. 1, Table 1) had a congenital anomaly in her right coronary

Table 1. Clinical data of the different patient groups: ischaemic heart disease (IHD) 1–7, atrial septal defect (ASD) 8–12, and valvular heart disease (VHD) 13–22. The main valvular lesion is italicized and the history of clinically overt congestive heart failure is expressed with CHF

Patient	Age, sex	Diagnosis	Duration of symptoms (years)	Degree of symptoms (NYHA criteria)	Drug treatment (mg/day)	Arrhythmias or disorders of conduction	Heart volume (cc/m^2)	Blood pressure (mm Hg)
1	31, ♀	IHD, coronary anomaly	4	I–II	Acenocoumarol 2	–	390	115/65
2	37, ♂	IHD	2	III	Alprenolol 300 Clofibrate 1500	–	375	140/80
3	43, ♂	IHD	3	II	Practolol 200 Clofibrate 1500	Left bundle branch block	570	115/75
4	49, ♂	IHD	2	III	Practolol 200	–	380	130/80
5	52, ♂	IHD, *AI*, AS, MI	3	III	Digoxin 0.125 Oxprenolol 120 Clofibrate 750	–	615	135/80
6	53, ♂	IHD	4	III–IV	Digoxin 0.250 Practolol 300 Clofibrate 1000 Warfarin 5–7.5	–	370	120/60
7	54, ♂	IHD, Hypertensio arterialis WHO I	6	III	Propranolol 320	–	500	140/80
Mean ±SD	46 9		3 1			Mean ±SD	455 105	115/75 35/10
8	19, ♀	ASD	–	–	–	–	550	105/70
9	25, ♀	ASD	3	I–II	Digoxin 0.250	–	430	115/75
10	25, ♀	ASD	–	–	–	–	490	130/80
11	31, ♀	ASD	–	–	–	–	530	130/90
12	49, ♀	ASD	4	I	Digoxin 0.250	AEB, VEB	760	115/80
Mean ±SD	30 12		–			Mean ±SD	550 125	120/80 10/10
13	22, ♂	*AI*, VSD, CHF	3	III	Digoxin 0.375	–	1100	180/60
14	40, ♂	*MI*, MS, AS, AI, CHF	7	IV	Digoxin 0.375 Frusemide 160 Spironolactone 75 Warfarin 4.5	FA	870	150/80
15	41, ♀	*MS*, MI, CHF	12	III	Digoxin 0.375 Frusemide 80 Warfarin 5	VEB	540	135/85

Table 1 (continued)

Patient	Age, sex	Diagnosis	Duration of symptoms (years)	Degree of symptoms (NYHA criteria)	Drug treatment (mg/day)	Arrhythmias or disorders of conduction	Heart volume (cc/m^2)	Blood pressure (mm Hg)
16	51, ♂	*MI*, AI, TI, CHF	10	IV	Digoxin 0.625 Frusemide 160 Hydrochlorothiazide 25 Amiloride 2.5	FA	1400	125/80
17	51, ♂	*MS*, MI	7	III	Digoxin 0.500 Spironolactone 50 Hydrochlorothiazide 50 Warfarin 5	FA	990	130/90
18	52, ♀	*MS*, MI, AS, AI, CHF	11	III	Digoxin 0.500 Frusemide 40 Spironolactone 50	FA	720	160/90
19	55, ♂	*MS*, MI, AS, AI, Arthritis rheumatoides	12	II	Digoxin 0.375 Frusemide 40 Chloroquine 40 Indomethacine 75	FA	900	130/80
20	57, ♂	*AS*, AI, IHD	2	II	Digoxin 0.250 Frusemide 40 Warfarin 5–7.5	Left bundle branch block	520	130/65
21	59, ♂	*MI*, MS, AS, AI	3	II–III	Digoxin 0.500 Hydrochlorothiazide 50	FA, VEB	1030	140/95
22	60, ♂	*AS*, Hypertensio arterialis WHO I	12	II	Digoxin 0.250 Chlorthalidone 50	Left bundle branch block, Dissociatio atrioventr. gr. I	500	125/90
Mean	49		8			Mean	855	130/80
±SD	12		5			±SD	290	40/10

AS = aortic stenosis; AI = aortic insufficiency; MS = mitral stenosis; MI = mitral insufficiency; TI = tricuspidal insufficiency; FA = atrial fibrillation; AEB = atrial ectopic beats; VEB = ventricular ectopic beats

artery and a fistulous connection between her main left coronary and pulmonary artery with resulting anginal symptoms, remaining cases had coronary atherosclerosis. In addition to IHD one patient (patient no. 5, Table 1) also had concomitant combined aortic valve lesion and slight mitral regurgitation.

Samples of the right auricle were obtained during open-heart surgery from the second group of five female patients with uncomplicated atrial septal defect of the secundum type (ASD). Their age varied from 19 to 49 years. The third group consisted of five male patients with a left-sided

univalvular or multivalvular heart disease (VHD) without clinically overt congestive myocardial failure, and five other patients, two females and three males, who had experienced congestive heart failure (CHF) based on VHD prior to surgery. Their age varied from 22 to 60 years. All the last mentioned patients with CHF showed a tendency to dyspnoea at rest, orthopnoea and an increase in venous pressure and body weight due to oedema. The degree of myocardial failure and physical performance were assessed according to clinical and laboratory findings and to the classification of the New York Heart Association (1964). It is quite obvious that the classification of the patients seriously ill with VHD into subgroups of those who have had, or are lacking, clinically overt congestive heart disease is somewhat arbitrary.

One patient of the VHD/CHF group (patient no. 20, Table 1) had had acute myocardial infarction two years previously.

Details of the patients of the various groups are presented in Table 1.

Drug Treatment

The usual daily medication (Table 1) was continued until surgery except for any beta-adrenergic blocking agents, which were withdrawn ten days previously. No drugs known to influence tissue catecholamine (CA) storage were used.

Fluorescence Histochemistry

A small wedge resection specimen of the right atrial tissue was excised from the auricular appendage at the insertion of the venous drainage perfusion cannula before the institution of cardiopulmonary bypass and operative correction. The resected specimens were immediately frozen in liquid nitrogen ($-196°C$) and stored until the whole of the material had been collected. Thereafter all the specimens were processed at the same time, in exactly the same manner, in order to assure a reliable comparison between different samples.

The glyoxylic acid fluorescence histochemical method (Lindvall and Björklund, 1974; Lindvall et al., 1974) was used in a slightly modified form (Waris and Partanen, 1975; Kyösola, 1976). The method has been found to be extremely sensitive and specific, and suitable also for studies on humans (Kyösola et al., 1976).

Specimens were removed from liquid nitrogen and immersed in ice-cold 2% glyoxylic acid (glyoxylic acid monohydrate, Fluka AG. Buchs SG, Switzerland) dissolved in 0.1 M phosphate buffer. The pH of the solution was adjusted to 7.0 with 1.0 N NaOH and it was kept ice-cold in beakers placed in crushed ice. After incubation for one hour, the endocardium was dissected with delicate forceps, the thin myocardial tissue strips were then torn off and further incubated in the glyoxylic acid solution for five more minutes. These strips were then dried with blotting paper, stretched on microscope slides, dried in a current of hot air for 15 min, heated in an oven at 100°C for six minutes, mounted in a mixture of xylene and Entellan® (E. Merck, Darmstadt, West Germany) and covered with a cover slip.

The specimens were examined and photographed with a Leitz-Wetzlar fluorescence microscope equipped with an epi-illuminator (Ploem, 1971). A HBO 200 high-pressure mercury lamp served as the ultraviolet light source. The filter combination was BG 38 (4 mm), $2 \times$ BG 3 (each 3 mm) and TAL 405 (Schott & Gen., Mainz, West Germany) as the primary filters and K 470 as the secondary filter.

From each specimen a large number of stretch preparations on several microscope slides were made. All the stretch preparations were analyzed one after another, in series, independently by three investigators without any knowledge of the respective patient. The analysis was repeated after about two months, with the specimens stored in darkness at $-40°C$ in the meantime. In analyzing the fluorescence histochemical characteristics of the specimens, particular emphasis was laid on the following criteria:

Table 2. Classification of the findings concerning the adrenergic structures in the atrial samples from patients of various groups of heart diseases. Ischaemic heart disease (IHD) 1–7, atrial septal defect (ASD) 8–12, and valvular heart disease (VHD) 13–22. For explanation of the symbols see Methods

Patient	Intensity of fluorescence	Density of adrenergic fibres	Size and number of varicosities per given length of adrenergic axon	Proportional amount between perivascular/myocardial adrenergic fibres	Amount of connective tissue fibres	Droplet fibres
1	+++	+++	+++	+++/+++	++	−
2	+++	+++	+++	+++/+++	++	−
3	+++	+++	+++	+++/+++	+	−
4	+++	+++	+++	+++/+++	++	−
5	+	+	++	+/+	++	−
6	++	++	++	++/++	+++	+
7	+++	+++	+++	+++/+++	++	−
8	++	++	++	++/++	++	−
9	+	+	++	+/+	+	−
10	+++	++	+++	+++/+++	++	−
11	++	+	++	++/++	++	−
12	++	++	+++	++/++	+	−
13	++	++	+++	++/++	+	+
14	+	0	0	0/0	+++	++
15	+	+	+	+/+	++	−
16	+	0	0	0/0	+++	++
17	+	+	+	+/+	+++	+
18	++	++	++	++/+	++	+
19	+	+	+	+/+	+++	−
20	+	0	0	0/0	++	−
21	+	+	+	+/+	+++	+
22	+	+	+	+/+	+++	−

1. Intensity of the fluorescence of the varicosities ("boutons") of comparable size taking into account separately the small, medium-sized and large varicosities. The results are depicted by +, ++, or +++, meaning weak, "average" or intense fluorescence, respectively.

2. The number of fluorescing nerves in a given area, i.e. the density of the visualized adrenergic network. The results of the estimation are expressed using 0 (=most specimens devoid of visualizing adrenergic nerves), + (=scarce distribution of fluorescing nerves), ++ (="average" number of fluorescing nerves), and +++ (=abundance of fluorescing nerves).

3. The average size of the visualized varicosities, the number of varicosities per given length of axon, and the proportional share of the large varicosities. Each of these criteria was checked independently, after which the results were summarized and expressed using 0 (=few tiny varicosities scattered at relatively long intervals), + (=some small and medium-sized varicosities), ++ (= "average" amount of different sized varicosities), and +++ (=abundance of large varicosities).

4. The proportional density between the perivascular nerve plexuses and the three-dimensional myocardial loose-meshed nerve net, obviously not related to the blood vessels. The densities of the innervation are expressed as in Chapter 2.

5. The amount of connective tissue fibres between bundles of myocardial cells is expressed arbitrarily using + (=scarce), ++ ("average"), or +++ (=abundant).

6. Attention was also paid to "droplet fibres" (Dahlström, 1970) expressed using +(=some) or ++ (=several) as well as to the relative amount of lipofuscin, which appeared in droplets and aggregates of varying size fluorescing orange to red.

The relevant data are presented in Table 2.

Results

ASD

Following the course of blood vessels, typical perivascular nerve plexuses were observed, consisting of fluorescing varicose axons and small fascicles of these, as well as occasional large nerve trunks (Figs. 1b and 2c). In all probability some of these axons represented the adrenergic innervation of the blood vessels themselves, while others, surrounding the vessels more loosely, were destined to innervate other target structures. In fact, single fluorescing axons and small fascicles of these were observed to leave the perivascular plexuses at intervals, thus contributing to the three-dimensional loose-meshed nerve net within the myocardial layer (Fig. 1e). This net mainly consisted of single varicose fluorescing axons or small fascicles containing a few individual axons closely abutting onto or coiling around each other. Sometimes large nerve fascicles or even compact nerve trunks were observed. At high magnification it was apparent that most of these nerve fascicles also included non-fluorescing fibres, probably cholinergic axons.

The varicosities were of varying sizes. In general, most of these synaptic enlargements in the ASD group were of medium size (Figs. 1b and e, 2c), the small and large varicosities being in the minority. The varicosities were generally rounded and exhibited an average fluorescence intensity, the intervaricose portions of the axons were not visualized. The specific NA fluorescence of the adrenergic varicose axons was readily discriminated by the blue-green to bluish colour, by the high intensity of fluorescence, and by the distinct contours of the varicosities from the non-specific fluorescence of the connective tissue (Fig. 2d). The latter was predominantly of yellow-green to greenish hue and of much weaker intensity. Lipofuscin contained within the myocardial cells, concentrated mainly near the poles of the ovoid nucleus, appeared as orange to red droplets or aggregates of varying sizes and shapes, readily visible against the weak homogeneous greenish background fluorescence of the sarcoplasm. For further comparison between this group and the IHD and VHD/CHF groups see Table 2.

IHD

In principle the pattern of the intrinsic innervation of the myocardium was similar in this group to that in the ASD group. Some clear-cut differences were obvious. The densities of both the perivascular and myocardial nerve nets were greater in the IHD group compared with those in the ASD group (Figs. 1a and 2a). Aso in this group, the average size of varicosities, the number of varicosities per given length of axon, and the proportional share of large varicosities were greater (Figs. 1a and 2a). The varicosities tended in many cases to be somewhat elongated and the intervaricose portions of the axons were also frequently visualized, a fact that obviously contributed to the elongated appearance of varicosities (Fig. 2a). The relative density of the perivascular

and myocardial adrenergic nerve nets was unchanged. Differences in fluorescence intensity between IHD and ASD groups could not be detected with certainty.

In one patient of this group (patient no. 6, Tables 1 and 2), "droplet fibres" were observed (Fig. 1 d).

Specimens from the patient with a coronary artery anomaly (patient no. 1, Tables 1 and 2) showed a picture identical to that of others of the IHD group, whereas the patient who had previously experienced myocardial infarction and had concomitant VHD (patient no. 5, Tables 1 and 2) showed a somewhat different picture closely resembling that seen in the VHD/CHF group (see below). For a more detailed comparison, see Table 2.

VHD/CHF

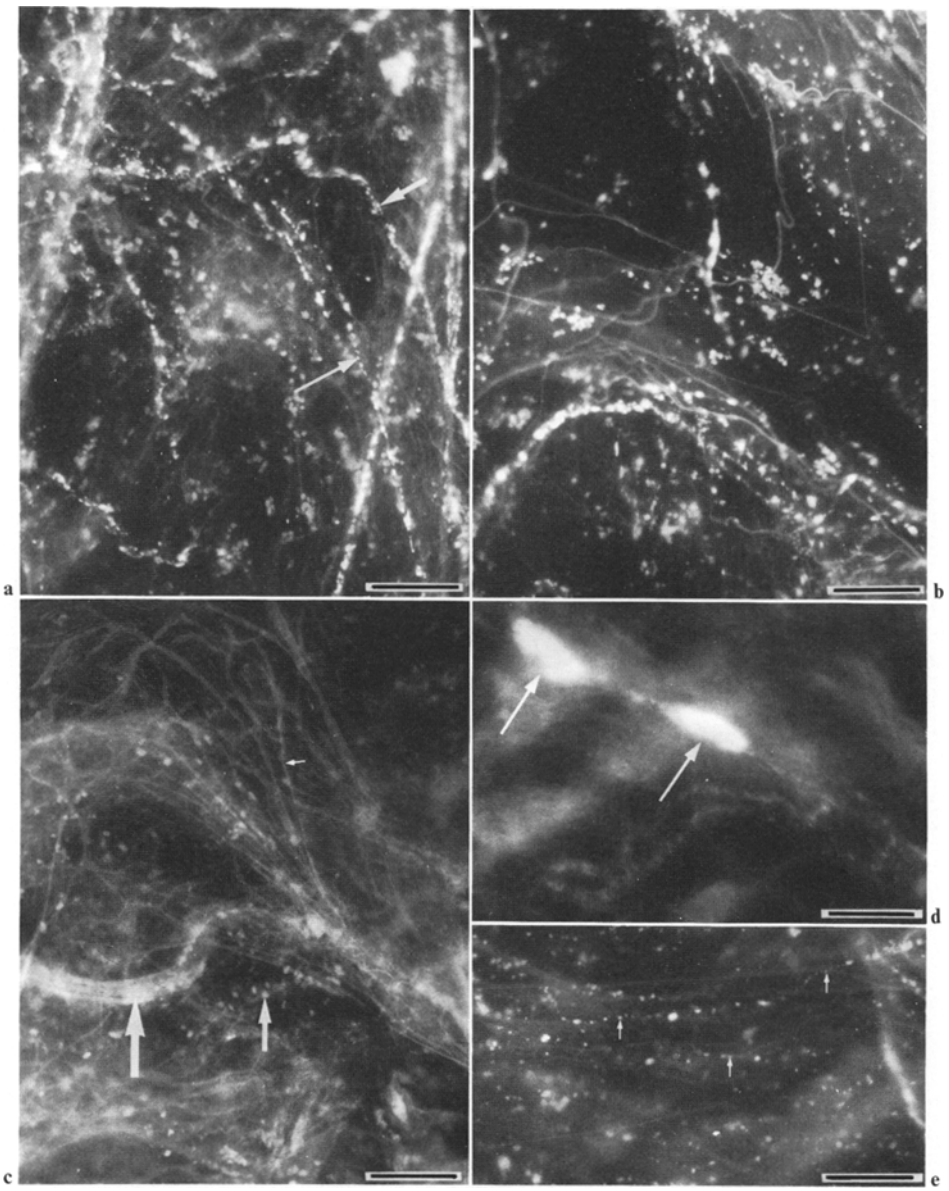
This group showed less fluorescence than the ASD group, assessed by the criteria of the density of the adrenergic nerve net, the intensity of fluorescence and the average size of varicosities (Figs. 1 c and 2 b). In some preparations, fluorescing axons were observed only occasionally, and the varicosities were very small and exhibited a hardly discernible fluorescence. The proportions of the perivascular and myocardial nerve nets apparently remained unchanged, although an impression was gained that the myocardial nerve net disappeared first and became depleted of NA to such an extent that it could no longer be visualized histochemically (Fig. 1 c). The overall impression was that the amount of NA in the adrenergic fibres in those patients of the VHD/CHF group is invariably less than that in the IHD and ASD groups. However, in some patients the adrenergic network was relatively well visualized with axons of ordinary fluorescence intensity and varicosities varying from small to medium size.

The "droplet fibres" (Fig. 1 d) were observed in six patients. The mean heart volume (cc/m^2) $\pm$ SD of all the patients with the "droplet fibre" phenomenon was 926 ± 323 ($n=7$) while that in the rest of the patients without such a finding was 537 ± 141 ($n=15$). The difference is statistically significant ($P<0.001$). Five of these seven patients with "droplet fibres" had atrial fibrillation.

For a more detailed comparison, see Table. 2.

Figs. 1 and 2. Glyoxylic acid–induced catecholamine fluorescence in the stretch preparations taken from the right atrial appendage of adult human heart. In addition to the specific catecholamine fluorescence, lipofuscin granules and connective tissue fibres exhibit an unspecific autofluorescence. The dark line on a white background in the lower right corner of each micrograph indicates 10 μ

Fig. 1. a Large numbers of strongly fluorescent adrenergic nerves from the heart of a patient suffering from IHD (patient no. 3, Table 1). Fluorescent varicose fibres run singly (long arrow) or course for long distances in close proximity to each other (short arrow). **b** Specifically fluorescent adrenergic nerves from the heart of a patient with uncomplicated ASD (patient no. 10, Table 1). The number of fluorescent nerves is slightly smaller than in the heart of the patient with IHD, see Figure 1 a. **c** The picture demonstrates the absence of the fluorescent nerves in a large area



of myocardium from the heart of a patient suffering from VHD and clinically overt congestive heart failure (patient no. 14, Table 1). In the centre of the picture is a blood vessel without fluorescent nerves. Quite intense autofluorescence is seen in the connective tissue fibres in the myocardium (small arrow), in the wall of the blood vessel (big arrow) and lipofuscin granules (medium-sized arrow). **d** Large, strongly fluorescent "droplet fibres" (arrows), diameter of the varicosities about 10 μ, from the heart of a patient suffering from VHD (patient no. 21, Table 1). Compare the size of the droplets with the size of the varicosities of normal adrenergic axons in Figure 1e (small arrows). **e** Single axons with many fluorescent varicosities of different size (small arrows) between the muscle fibres in the heart of a patient suffering from uncomplicated ASD (patient no. 8, Table 1)

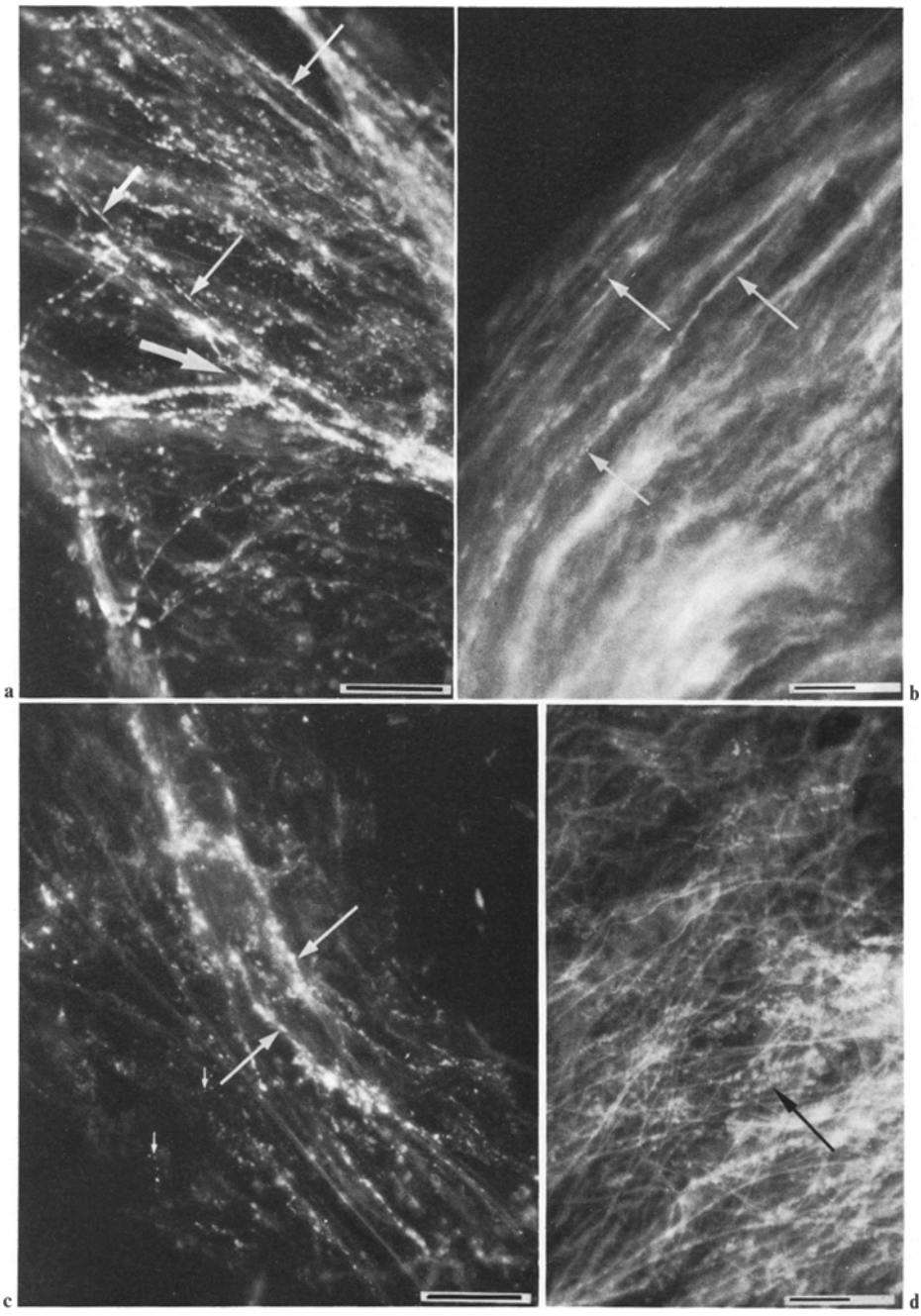


Fig. 2. a Large numbers of strongly fluorescent adrenergic nerves with numerous varicosities (long arrows) from the heart of a patient suffering from IHD (patient no. 4, Table 1). Both rich perivascular and myocardial adrenergic nerve nets can be observed. Sometimes the intervaricose portion is visible (short arrow). Compare with Figure 2c. The ramification of a blood vessel can be seen (big arrow). **b** A few adrenergic fibres (arrows) are seen between the abundant autofluorescent connective tissue bundles. The specimen was obtained from the heart of a patient suffering from VHD with clinically overt congestive heart failure (patient no. 13, Table 1). **c** A strongly fluorescent perivascular adrenergic plexus (long arrows) and single myocardial axons with many varicosities mainly of minimal size and intensity (small arrows) from the heart of a patient with uncomplicated ASD (patient no. 11, Table 1). In the myocardial axons the intervaricose portion is not visible. **d** Single fluorescent varicose adrenergic axons (arrow) between the very abundant autofluorescent elastin fibres. The specimen was obtained from the heart of a patient with ASD (patient no. 12, Table 1)

Discussion

In the present comparative study the following criteria were used in estimations: the intensity of fluorescence, the density of the adrenergic network, the median size of the varicosities and the relative proportions of the large varicosities, and the presence of the "droplet fibres".

Several methods have been used for the quantitation of the fluorescence (induced by formaldehyde incubation) of the biogenic amines (for ref. see Jonsson, 1969; Lidbrink and Jonsson, 1971; Jonsson, 1971; Jonsson, 1974; Einarsson et al., 1975). It has been found that the intensity of fluorescence is proportional to the NA concentration up to a value corresponding to about 30–40% of the endogenous NA level in the adrenergic axons. When the nerves contain more than 40% of the endogenous NA level, there is no corresponding increase in the fluorescence intensity, probably because of a concentration-dependent quenching of fluorescence. This means that the NA content has to be lowered by at least 60% in order to be able to detect a decrease in the fluorescence intensity (Jonsson, 1971). In the area of the linear relationship, the direct visual fluorescence intensity estimation in fluorescence microscopy is a fairly easy procedure for a trained and experienced microscopist, and one with a good degree of reproducibility (Lidbrink and Jonsson, 1971). The main problem in using the present method for monoamine quantitation is that local intraneuronal concentration of monoamine is generally so high that a concentration-dependent quenching of fluorescence exists. The situation is further complicated by the fact that the fluorescence yield is affected by the subcellular localization of monoamine, the fluorescence yield being higher when the monoamine is distributed extragranularly. However, it can be concluded that if changes in the fluorescence intensity are recorded as compared with a control, this reflects true changes in amine concentration. In doubtful cases the fluorescence estimations should be correlated with biochemical determinations of monoamine content in comparable pieces of tissue (Jonsson, 1971). Such estimations of NA content from right auricular tissue in respective patient groups were made and significant differences were found. In the IHD group the NA content ($\mu g/g \pm SD$) was 2.81 ± 0.94 ($n=19$), in the uncomplicated ASD group 1.64 ± 0.32 ($n=10$) and in the CHF group 0.75 ± 0.41 ($n=11$) (Penttilä et al., 1975, 1977). Histochemically the difference in fluorescence intensity was obvious between the groups of IHD and VHD/CHF and ASD and VHD/CHF but not between the groups of IHD and ASD, which is probably due to the quenching phenomenon.

Subjective estimation of the fluorescence intensity in fluorescence microscopy has been used extensively for the semiquantitative estimation of neuronally-stored monoamines under various experimental conditions and it has been found that the human eye has a very high capacity for distinguishing between two areas having very small differences in brightness, provided that they have the same colour (Jonsson, 1971). In estimating and comparing fluorescence intensities, it is important, however, that the illumination of the specimens is kept as constant as possible from one specimen to another.

The estimation of the number of nerves or the nerve density is most easily performed in tissues with an evenly distributed network, e.g. the iris and vas

deferens. Our opinion is that in this respect conditions are comparable to those in human right auricular myocardium. In such tissues it is possible to detect changes in the nerve density. There is generally a good correlation between the nerve density as recorded histochemically and the amount of NA as recorded by chemical assay (Jonsson, 1971).

This histochemical study supports the results obtained by a biochemical technique of a high NA content in human auricular tissue in IHD (Penttilä et al., 1975, 1977). Compared with the VHD/CHF group, NA fluorescence in IHD tends to be more intense, the varicosities larger and more numerous and the adrenergic network visualized more dense. The situation tends to be parallel in the ASD group compared with the IHD group although neither the density of the adrenergic network nor the proportional share of the largest varicosities were as high or the intervaricose part of the axon as clearly visualized as in the IHD group.

In the VHD/CHF group it is that component of the adrenergic network running freely in the myocardium, independent of blood vessels in which the fluorescence intensity and density of fluorescing fibres, are primarily diminished. These findings are in agreement with histochemical observations on failing bovine heart (Vogel et al., 1969).

Earlier a positive correlation between auricular and ventricular NA concentrations has been shown to exist (Chidsey et al., 1965). Accordingly it may be assumed that in IHD the ventricular NA level is also rather high and the adrenergic network rather dense. CA produces an increase in the myocardial contractility and stimulation of metabolism (Barrett and Einstein, 1975). In ischaemia the release of stored NA has been suggested as playing a key role in the initiation of the intracellular metabolic response, resulting in impaired contractility, arrhythmias and changes in membrane permeability. This release takes place within a few seconds of the onset of ischaemia (Schahab et al., 1972; Oliver, 1975). This study also supports the view that both in normal conditions and in IHD there are good local facilities for increasing the sympathetic drive when necessary.

There were differences in medication between the groups but no drugs known to interfere significantly with the tissue CA content were used. All but one of the patients of the IHD group had used beta-adrenergic blocking drugs, which, however, were regularly withdrawn ten days before the operation. This time clearly exceeds the limit for complete recovery from the cardiac effects of beta-adrenergic blocking agents or any of their active metabolites (Carruthers et al., 1973; Faulkner et al., 1973). In an animal experiment (Klinge and Aro, 1971) no alteration could be shown in the NA content of rat heart after treatment for a week with high doses of commonly used beta-adrenergic blocking agents. It remains to be seen whether the high concentration of NA in IHD is connected with the withdrawal syndrome caused by the cessation of therapy with beta-adrenergic blocking agents (Miller et al., 1975). Perhaps this is a question of receptor hypersensivity (Malagelada et al., 1974) in connection with the cessation of the chemical denervation of the target organ.

It is well known that after a peripheral adrenergic nerve is cut, large amounts of NA, easily detectable in fluorescence microscopy, accumulate on the proximal

side while only minimal amounts, if any, remain on the distal side of the nerve. Because of the accumulated NA, the axons may became enlarged and bulge (Dahlström, 1970). In the present work fluorescing axons of identical appearance, here called "droplet fibres", were observed in seven samples, six of which represented patients with VHD and one with IHD. The mean heart volume was significantly higher in these patients than in the rest of the patients. Thus, the occurrence of "droplet fibres" may be related to hypertrophy of myocardium with consequent mechanical trauma with stretching or even disruption of adrenergic axons. It may be assumed that these local accumulations of NA are relatively common in patients of this kind and could constitute one extra contributory factor to the tendency to arrhythmias, by increasing the excitability of non-automatic tissue. It is interesting that the majority of the patients with the "droplet fibre" phenomenon had atrial fibrillation. Further, it may be suggested that such axonal destruction may lead to variable denervation of the myocardium and to an increase in the role of circulating CA.

The glyoxylic acid-induced fluorescence histochemical method (Lindvall and Björklund, 1974; Lindvall et al., 1974) seems to be suitable for comparative studies in human clinical material.

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