Catecholamine Content and Uptake of the Ductus Arteriosus of the Fetal Lamb

Recent morphological studies on innervation of the ductus arteriosus revealed both adrenergic and cholinergic nerves in the vessel wall of the ductus and suggested the possibility of some neural control[1–5]. Based on these evidences, quantitative comparison of nerve density in the ductus and adjacent vessels was carried out by fluorometric determination of endogenous catecholamine (CA) and by uptake measurement of ^{14}C-noradrenaline (^{14}C-NA) in the vessel wall.

Methods. Theductus and adjacent portion of the pulmonary trunk and aorta were removed by the method described elsewhere[3]. A fluorometric procedure was used to determine endogenous CA as described by FLEMING et al.[6]. For ^{14}C-NA uptake studies, thin, cross-sectioned slices of the arteries were prepared, weighed (75–240 mg) and placed in 4.8 ml of Krebs-Ringer bicarbonate medium[7] containing 0.2 mg/ml of ascorbic acid. They were incubated with ^{14}C-NA (0.1 µg base/ml) in a 5% CO_2-95% O_2 atmosphere at 37°C for 30 min. After incubation, the tissues were rinsed twice, 5 min each in ice-cold medium and homogenized in 1.7 ml of 2 *N* acetic acid. Extraction and separation of NA from metabolites on an alumina column were accomplished as described previously[8]. Aliquots of the fractions were counted in a Packard Tri-Carb. Uptake of radioactivity is expressed as the tissue-to-medium ratio which is the disintegrations per min/g of tissue divided by the disintegrations per min/ml of medium.

Results. Determinations of CA on vessels from 20 fetal lambs are shown in Table I. The pulmonary end of the ductus had more NA than its aortic end ($p < 0.01$). There was, moreover, a concentration gradient from within the pulmonary trunk through to, and including the aorta. The content of dopamine in the ductus exceeded that of NA ($p < 0.01$), while the reverse held true in the right atrium ($p < 0.05$).

Radioactivity in the vessels rose to about 3–5 times that in the medium (Table II). Analysis by column chromatography revealed that about half of the total radioactivity present in the vessels after incubation was in the form of unchanged NA. The tissue-to-medium uptake ratio of labelled (unchanged) NA in the pulmonary end of the ductus was significantly higher than that in the aortic end of the ductus ($p < 0.01$). The tissue-to-medium uptake ratio in the pulmonary trunk was higher than that in the aorta ($p = 0.05$).

Discussion. Our chemical measurement of CAs in the vessel walls indicated an amount of endogenous NA in the ductus comparable with the concentration of 0.14–0.70 µg/g in the peripheral arteries of the forelegs of the dog[9] which are under physiological neural control; this is in contrast to the concentration of 0.005 µg/g in the extra-abdominal part of the human umbilical artery[10] which lacks innervation. If there is close correlation between the CA content and the response to adrenergic neural stimulation[11], the ductus possesses enough neurotransmitter to respond to stimulation of its adrenergic nerves.

The conditions of our analysis indicate that the uptake of ^{14}C-NA is mainly by nerve endings rather than by extra-

Table I. Catecholamine content of arterial segments and heart of fetal lambs

	Dopamine[a] (µg/g)[c]	Norepinephrine[b] (µg/g)[c]
Proximal pulmonary trunk	1.02±0.22	0.53±0.05
Distal pulmonary trunk	1.03±0.13	0.50±0.05
Pulmonary end of the ductus	1.43±0.33	0.44±0.11
Aortic end of the ductus	1.58±0.42	0.27±0.05
Proximal aorta	0.61±0.06	0.39±0.05
Distal aorta	0.54±0.05	0.29±0.04
Right atrium	0.61±0.10	0.75±0.09

[a] Mean ±S.E. of 3 trials of analysis on tissues from 6 lambs. [b] Mean ±S.E. of 10 trials of analysis on tissues from 20 lambs. [c] µg/g wet weight tissue.

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Table II. Tissue-to-medium catecholamine uptake ratios derived from vessels of fetal lambs (mean±S.E. of 4 trials of analysis on tissues from 4 lambs)

	Total T/M[a]	NE T/M[b]	Relative T/M[c]
Proximal pulmonary trunk	4.97±1.10	2.79±0.97	145.8±20.1
Distal pulmonary trunk	4.06±0.37	2.41±0.42	138.3±12.5
Pulmonary end of the ductus	4.08±0.54	2.37±0.42	134.3± 9.8
Aortic end of the ductus	3.01±0.36	1.77±0.36	100.0
Proximal aorta	3.89±0.53	2.70±0.49	117.5±18.7
Distal aorta	3.22±0.27	1.74±0.27	101.4±11.9

[a] Tissue-to-medium uptake ratio of total radioactivity. [b] Tissue-to-medium uptake ratio of nonmetabolized norepinephrine. [c] Percentage uptake of non-metabolized norepinephrine relative to aortic end of the ductus which was taken as 100.0.

neuronal sites[12]. Provided that nerve endings in the pulmonary trunk, ductus, and aorta are similar in their storage and uptake abilities, both the endogenous CA content and the uptake ratio would reflect the density of nerves in these vessels.

The significance of the relatively high concentrations of dopamine in the ductus of fetal lamb, as compared with the human ductus where no measurable dopamine was found[4], is unknown.

Contrary to the visual estimate of our earlier observation[3], the quantitative estimate of CA content and NA uptake revealed both to be consistently higher at the pulmonary end of the ductus than at the aortic end. The presence of this gradient along the ductus does not, however, necessarily imply a specific role of adrenergic innervation in function of the ductus alone, since this is only part of a larger gradient extending from within the pulmonary trunk through to, and including the aorta. Since KENNEDY[13] stressed the importance of a rise in arterial Po_2 as a major determinant of closure, this hypothesis has been substantiated by many studies on the ductus of the human[14,15] and of animals[16–18]. However, the possible interrelations between effects of oxygen and those of innervation were not clarified, e.g., whether the response to raised Po_2 was diminished after denervation.

A number of experiments have favored the involvement of a nervous mechanism in the closure of the ductus. The autonomic innervation of the fetal heart and blood vessels is readily responsive to stimuli[19–23].

Taken together, these several pieces of evidence suggest that adrenergic innervation of the ductus may play a role in closure of the ductus auxiliary to that played by arterial Po_2. The possible role of the previously demonstrated cholinergic innervation[5] must also be investigated[24].

Zusammenfassung. Die Verteilung von Noradrenalin und Dopamin im Ductus arteriosus des Lammes wurde studiert und mit ^{14}C-Norepinephrin die Fähigkeit, exogenes Noradrenalin aufzunehmen, geprüft.

M. IKEDA[25], R. R. SONNENSCHEIN and D. T. MASUOKA

Department of Physiology, University of California, School of Medicine, Los Angeles (California 90024, USA), and Neuropharmacology Research Laboratory, Veterans Administration Hospital, Sepulveda (California 91343, USA),
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[25] Department of Cardiovascular Surgery, Kenritsu-Amagasaki Hospital, Amagasaki, Hyogo-ken (Japan).

Cardiac Output in Thyroid Disease

Concomitant cardiovascular disorder in thyroid disease is a well known fact since the early description of MOEBIUS in 1896[1]. Thyroid hormone possesses a positive chronotropic and inotropic action. Heart rate, maximum velocity of fiber shortening, rate of tension development and cardiac output are therefore increased. In hypothyroidism these parameters are all reduced[2,3]. Decreased peripheral vascular resistance is another important factor in thyroid-induced high output state. The effects of thyroid hormone seem to be independent of the norepinephrine stores of the heart[2,4].

Increased oxygen consumption alone does not explain the elevated cardiac output in hyperthyroidism, and the augmentation of cardiac output, when compared to oxygen consumption, is in excess of that observed during exercise[5]. Nevertheless some relationship between cardiac output and oxygen consumption seems to exist. The purpose of this study was to investigate the correlation between basic metabolic rate (BMR) and cardiac output.

Material and methods. 24 patients with clinical evidence for thyroid disease and 6 normal controls were investigated. Thyroid function studies including BMR (according to method of Harris) and tracer studies with I^{131}[6] were done in all and protein-bound iodine determinations (PBI) were done in 22 protein. Within 5 days right heart catheterization with microcatheters was performed by previously described methods[7] and right atrial-, right ventricular- and pulmonary artery pressures were measured. Cardiac output was calculated according to the Fick prin-

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