Bayliss myogenic response in the isolated ductus arteriosus of guinea-pig and rabbit fetuses

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Summary. Isolated ductus arteriosus responds by marked constriction to increases in perfusion pressure. If, however, these increases exceed 90 mm Hg–130 mm Hg, the vessel suddenly dilates and its responsiveness becomes depressed. The importance of these findings in postnatal closure of ductus arteriosus is discussed.

Increase in arterial pO_2 is generally thought to play the main role in closure of ductus arteriosus (DA)[1]. Under certain specific conditions, however, several other factors may become critical[2,3]. Thus, asphyxia or ligation of umbilical vessels leads to an increased arterial pressure in the newborn infant[4,5]. This increase in pressure unavoidably results in DA wall distension which in turn may evoke a constrictoric response based on the Bayliss' mechanism[6].

We tried to verify experimentally the assumtion that in DA it would be possible to evoke myogenic response, i.e. this vessel would respond to changes in transmural pressure by an active change in its diameter.

Material and method. Experiments were made in DA of guinea-pig and rabbit fetuses (22 and 20 preparations, respectively). Fetuses were obtained by cesarean section at the expected date of birth, and they were killed by dissection of their carotic arteries. Hearts with great arteries were prepared, a cannule was inserted into the pulmonal artery, and the DA was cut off at its aortal end[7]. The isolated vessel segment was placed to a muscular chamber and perfused with modified nitrogen bubbled Tyrode's solution, using perfusion pump[8]. 2 air-reservoirs linked with Starling's resistance permitted sudden changing of perfusion pressure. In several experiments, single doses of 10–100 µg noradrenaline were applied through tubing above the preparation.

Results. In 39 DAs the perfusion pressure was suddenly raised from 35 mm Hg to 75 mm Hg in average; 82% of preparations responded by a 2-phase change in flow through DA. After a short initial increase, the flow gradually decreased and reached a steady state at a new lower level. Thus, the DA flow resistance increased. No significant differences in this response were observed between guinea-pig and rabbit DAs.

Figure 1 is an original record representing the 2 characteristic types of the responses observed. Figure 1, A: A transient increase in flow followed by a relatively rapid decrease, reaching a steady state after 3–5 min. The DA flow resistance expressed in PRU units (mm Hg/ml/min) increased from the inital 0.835±0.085 to 1.614±0.166 (mean±SEM). Figure 1, B: Continuous flow decrease, steady state levels reached after 5 min at a relative low level (decrease in average from 78 ml to 5 ml/min). Such a pronounced constrictoric response was observed in 6 DAs.

In 7 experiments the perfusion pressure was gradually increased by 10 mm Hg in a range between 20 mm Hg and 200 mm Hg, and the respective flow through DA was recorded. Figure 2 summarizes the results of these experiments. As the pressure-flow curves show, 2 distinct sections can be distinguished: a) within the pressure range 20 mm Hg–90 mm Hg, the flow did not significantly change; b) within the pressure range 90 mm–130 mm Hg, the flow suddenly increased, to reach up to 4 times higher values than previously. When pressure kept increasing stepwise, the flow became significantly dependent upon pressure following this sudden dilation. At the same time (as the responsiveness to pressure impulses became diminished or even abolished), also responsiveness to pharmacological stimuli became significantly reduced (3–10 times higher doses of noradrenaline were required to reach minimum constriction).

Discussion. The finding of marked mechanosensitivity of DA[9] has already suggested the presence of myogenic responsiveness in this fetal shunt. Based on the above results, this suggestion can be considered as verified: DA responds

1 M.A. Heymann and A.M. Rudolph, Physiol. Rev. *55*, 62 (1975).
2 D.E. Cassels, in: The ductus Arteriosus, p. 75. Charles C. Thomas Publisher, Springfield 1973.
3 M. Ikeda, E.H. Rubinstein and R.R. Sonnenschein, Proc. Soc. exp. Biol. Med. *143*, 354 (1973).
4 G.S. Dawes, in: Foetal and Neonatal Physiology, p. 184. Year Book Medical Publisher, Chicago 1969.
5 G.V.R. Born, G.S. Dawes, J.C. Mott and B.R. Rennick, J. Physiol. *132*, 304 (1956).
6 W.M. Bayliss, J. Physiol. *28*, 220 (1902).
7 V. Kovalčik, J. Physiol. *169*, 185 (1963).
8 M. Kriška and V. Kovalčik, Folia Fac. med. Univ. Com. Bratisl. *11*, 145 (1973).
9 J.A. Kennedy and S.L. Clark, Anat. Rec. *79*, 349 (1941).
10 R.G. Gillman and A.C. Burton, Circulation Res. *19*, 755 (1966).
11 T.W. McIntyre, Biophys. J. *9*, 685 (1969).

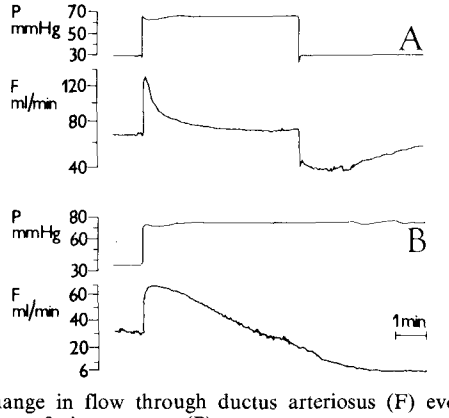

Fig. 1. Change in flow through ductus arteriosus (F) evoked by increase in perfusion pressure (P).

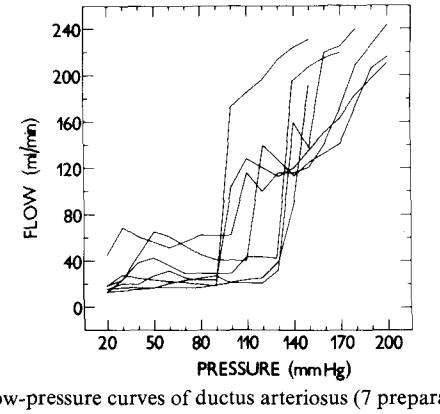

Fig. 2. Flow-pressure curves of ductus arteriosus (7 preparations).

to changes in transmural pressure by active changes in its diameter in the sense of Bayliss response. This myogenic mechanism very likely contributes to the postnatal closure of DA as a factor potentiating responses to increase in pO_2[3], and in extreme situations (e.g. fetal asphyxia) or in certain species with weakly reacting DAs to oxygen[8,10], it may even play the role of the main factor.

The observed sudden dilation of DA, associated with depression in its responsiveness when pressures 90 mm Hg-130 MM Hg are reached, may represent one of several pathogenic factors causing ductus arteriosus apertus. Such a dilation may be due to changes in histoarchitectonics[11], or ultrastructure in DA wall resulting from its distension to a critical degree.

Possible significance of aminotransferases in tissues of the aestivating fresh water mussel, *Lamellidens marginalis* (Lamarck)

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Summary. Both aspartate and alanine aminotransferase levels increased in digestive gland, foot and mantle on aestivation. The free amino acids and pyruvic acid also increased in all tissues. The significance of these changes is discussed in relation to gluconeogenesis.

Some species of fresh water gastropods[2-5] and pelecypodes[6] are known to aestivate under drought conditions. Studies on aestivation metabolism, mostly in gastropods, showed decrease in succinate dehydrogenase, cytochrome, C oxidase[7,8] and acetylcholine esterase[9].

Since the animals during aestivation desists from feeding and mainly depend on glycogen reserves, it is obvious that these reserves also deplete in course of time[2,5]. This necessiates the aestivating animal to depend on other sources for its survival. It is viewed that aminotransferase enzymes namely aspartate aminotransferase EC.2.6.1.1 (AAT) and alanine aminotransferase EC.2.6.1.2 (AlAT) to serve as link enzymes for carbohydrate and protein metabolisms[10]. Since these enzymes interconvert strategic compounds and act as sources for keto acids for Krebs' cycle and for gluconeogenesis[10], in the present study these enzymes were selected to ascertain its role during aestivation of fresh water mussel. Since the literature on aestivation in fresh water mussel is scanty, and no work has been done in fresh water mussels on these lines, an attempt has been made to correlate aminotransferase activity pattern with the aestivation phenomenon.

Materials and methods. The fresh water mussel (*Lamellidens marginalis* (Lamarck)) were collected from the ponds and adapted to laboratory conditions. They were fed with fresh water plankton. Aestivation was induced by keeping at a time 50 specimens in a glass troughs containing soil from the collecting spot and covered with little water. The water was allowed to evaporate at room temperature as suggested by Newman and Thomas[4]. Only 1 month aestivating specimens were selected for experimentation.

The AAT and AlAT activity levels in selected tissues were estimated by the method of Reitman and Frankel[11] after standardization. 3 tissues, viz. digestive gland, foot and mantle, were excised and homogenized in cold 0.25 M sucrose solution using Yorco tissue homogenizer (Yarco Scientific Industries, New Delhi) and centrifuged at 600× g. The supernatant was used for assay. The reaction mixture consists of 100 µmoles phosphate buffer (pH 7.4) 2 µmoles of α-oxoglutaric acid (pH 7.4) 20 µmoles of L-aspartate (pH 7.4) (for AAT); 50 µmoles of DL-alanine (pH 7.4) (for AlAT) and 0.1 ml of supernatant (10% w/v) as enzyme source. After incubating for 30 min, the reaction was arrested by adding 1 ml of 2,4 dinitrophenyl hydrazine (0.001 M in 0.1 N HCl). After 20 min, the colour was developed by adding 10 ml of 0.4 N sodium hydroxide solution. The colour was read at 546 nm in spectrophotometer model-CL-20 (Elico, India). The protein content was determined by using Folin Ciocalteu's reagent[12]. The free amino acid content was estimated by the method of

Levels of aspartate and alanine aminotransferases and organic acid. (Free amino acid and pyruvic acid) content in selected tissues of aestivating fresh water mussel. Each value is a mean of 6 individual observations ± SD

Enzyme/organic acids	Digestive gland		Foot		Mantle	
	Control	Aestivated	Control	Aestivated	Control	Aestivated
Aspartate aminotransferase*	5.075 ± 0.1766	6.925 ± 0.1893 + 36.45 p<0.001	3.01 ± 0.1546	5.05 ± 0.225 + 67.74 p<0.001	6.45 ± 0.447	7.0 ± 0.208 + 8.65 p<0.02
Alanine aminotransferase*	5.04 ± 0.171	7.015 ± 0.1995 + 39.18 p<0.001	3.62 ± 0.1393	4.82 ± 0.224 + 33.14 p<0.001	6.13 ± 0.2316	7.56 ± 0.2454 + 23.4 p<0.001
Free amino acids**	866.6 ± 30.76	1179.3 ± 46.05 + 33 p<0.001	1007.83 ± 25.16	1198 ± 27.2 + 18 p<0.001	390.3 ± 26.5	434.3 ± 24.46 + 11.5 p<0.001
Pyruvic acid***	0.1007 ± 0.0047	0.125 ± 0.0056 + 24.13 p<0.001	0.0412 ± 0.001509	0.0662 ± 0.006 + 60.67 p<0.001	0.0309 ± 0.0024	0.056 ± 0.0029 + 81.2 p<0.001

* Values expressed as µmoles pyruvate/mg protein/h. **Values expressed as µmoles tyrosine/g wet weight of tissue. *** Values expressed as mg pyruvic acid/g wet weight of tissue. +, indicates percent increase over control. p = t-test; all values are found to be significant.