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Altered response in vitro of the human umbilical artery to sera of neonates with respiratory distress syndrome and other hypoxic conditions

By

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A study was made of the reaction of the human umbilical artery and the isolated rabbit auricular artery to protein-free ultrafiltrates of normal and hypoxic sera originating from neonates suffering from RDS or other acute metabolic disturbances. While a normal serum ultrafiltrate caused an enhanced contraction of the umbilical artery, hypoxic sera exerted a weaker pressor activity. At the same time, on the rabbit's isolated auricular artery the hypoxic sera exhibited a higher pressor activity. The phenomenon is in agreement with the clinical observation that spontaneous closure of the umbilical vessels is delayed in the case of fetal hypoxia.

Study of the contractility and relaxation of the umbilical artery is of fundamental importance in neonatal pathology. Postnatal closure of the umbilical arteries is one of the most significant and frequent phenomena of the adaptation to extrauterine life. Under intrauterine conditions, regulation of vascular resistance is a vitally important mechanism for the fetus.

Interest in the reactivity of the umbilical artery has been promoted by the circumstances that a human organ preparation can be studied which does not contain nervous elements [11], and the constriction of which is regulated solely by vasoactive substances and metabolic effects. A characteristic feature of the umbilical artery is its sensitivity to changes of the pO_2; to its elevation the artery responds with contraction. The response is potentiated by humoral mediators; experimental studies have revealed the possible role in it of acetylcholine, serotonin, noradrenaline and bradykinin [9, 12]. Recently, the prostaglandins have come into the centre of attention, after it had been shown that the umbilical artery responds sensitively to the prostaglandin endoperoxides, and that these vasoactive substances are produced in the vascular wall itself in the course of contraction under the effect of O_2 [13, 16].

The reactivity of the umbilical artery has many features in common with the ductus arteriosus. Both types of vessel have been the subject of extensive investigations [10, 15]. The clinical observations of Desmond et al. [6] and our animal experiments

have revealed that closure of the umbilical artery is protracted postnatally following fetal hypoxia [3] and even fails to occur after oxygenization of the blood following prolonged hypoxia. Failure of the fetal vessels to contract is therefore a consequence of a humoral effect. The aim of the present investigation was to study whether the humoral effect due to hypoxia would affect the umbilical arteries under conditions *in vitro*.

Materials and Methods

Study of the humoral effect of serum developing in consequence of hypoxia was carried out with ultrafiltrates of sera of healthy blood donors of neonates with RDS (10 cases), and of infants and children with life-endangering conditions (10 cases). Blood was usually obtained by heart puncture immediately after death, while in 5 cases it was taken during life in a grave condition. The seperated sera were freed from protein by ultrafiltration through a UM-2 membrane on an Amicon Diaflo apparatus, and adjusted to pH 7.4 immediately before use. The umbilical cords were placed after birth into modified Krebs solution at room temperature, and used within one hour. Composition of the modified Krebs solution was NaCl, 6.90 g/l; $NaHCO_3$, 1.90 g/l; KCl, 0.20 g/l; KH_2PO_4, 0.16 g/l; $CaCl_2$, 0.20 g/l; $MgSO_4$, 0.13 g/l; and glucose, 1.00 g/l. The pH was adjusted with HCl to 7.4 before use. In order to avoid damage and traction of the artery, material taken from the central part of the umbilical cord was suspended in an organ bath at 37 °C and then examined in a perfusion system by the method developed by Kovalčik for the reactivity of the ductus arteriosus in experimental animals [10]. A stream of modified Krebs solution, most frequently with a pO_2 of 150 mm Hg, corresponding to atmospheric air, was passed over the organ at a rate of 4 ml/min. The vascular reaction was studied by measurement of the perfusion pressure with a Hellige electromanometer, and recorded with a Kutesz compensograph. After the pressure had become constant following the perfusion, the reactivity of the vessel was judged by its response to 50 μg of acetylcholine. Subsequently, always after testing with normal serum ultrafiltrate, the same amounts of pathological serum ultrafiltrate were added to the system for comparative purposes. 0.2—0.5 ml quantities of the serum ultrafiltrates were injected into the perfusion fluid at the site of influx into the organ. The reactivity of several materials was investigated on one preparation. After the tests, the reactivity of the vessel to normal serum ultrafiltrate and to acetylcholine was again checked.

In order to compare the effects exerted on the umbilical artery, the same materials were tested on a systemic artery. In these tests, the plasma pressor activity was observed on the isolated rabbit auricular artery obtained by the method of de la Lande [5].

Statistical analysis was performed by Wilcoxon analysis and by the single-sample t test.

Results

Results are shown in Fig. 1. It can be seen that the normal serum ultrafiltrate regularly exerted a pressor activity on the umbilical artery. The extent of its contraction induced by normal serum ultrafiltrate varied within wide limits, but even when this was taken into account, the serum of RDS patients had a much weaker effect. The difference was significant statistically ($P < 0.05$).

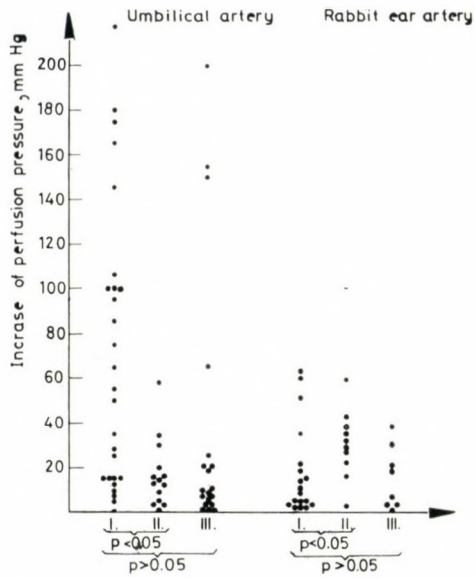

FIG. 1. Increase in perfusion pressure in the human umbilical artery and the isolated rabbit central auricular artery under the effect of protein-free ultrafiltrates of sera originating from normal neonates (I), neonates with RDS (II) and patients with other hypoxic conditions (III)

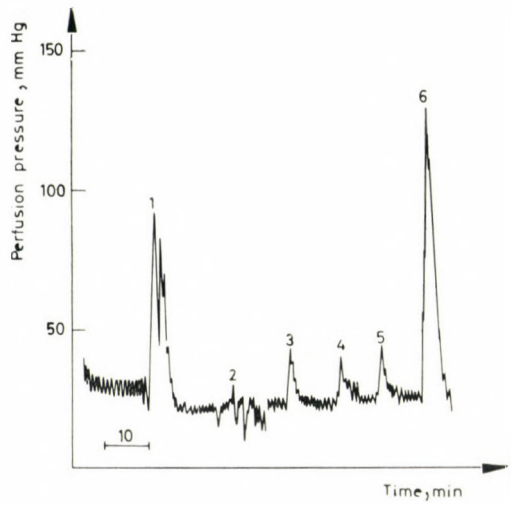

FIG. 2. Recorded curve of pressure changes in the umbilical artery, due to 0.5 ml serum ultrafiltrate from normal individuals (1 and 6), a neonate with RDS (4), and patients who were hypoxic as a consequence of malignant tracheobronchitis pneumonia (2 and 3). Modified Krebs solution pH 7.45, with a pO_2 of 150 mm Hg, passed at a rate of 4 ml/min. Perfusion pressure change due to 50 μg of acetylcholine (5)

The effect of other hypoxic serum ultrafiltrates was also weak, although to a lesser extent than that of RDS serum. This difference too was significant ($P < 0.05$).

The pressure curve recorded in a typical test is presented in Fig. 2.

On the rabbit auricular artery preparation, the same serum ultrafiltrates had an opposite effect. Normal serum ultrafiltrate often failed to cause constriction while RDS serum ultrafiltrates had an enhanced pressor effect. The change was significant statistically ($P < 0.05$). The effect of serum ultrafiltrates from other hypoxic patients was not significantly different from that of normal serum.

Discussion

It is characteristic of the umbilical artery that it reacts with intensive contraction to normal serum ultrafiltrate. The vasoactive property of normal serum [9] is well-known. Some of the reactive materials are bound to dialysable substances of low molecular weight, which are intensely potentiated by proteins. In order to exclude this latter effect, we carried out the test with protein-free ultrafiltrates. It appears that the umbilical artery is very sensitive to the vasoactive substances in normal serum and it was therefore particularly striking that its contractility decreased under the effect of hypoxic serum ultrafiltrate. This was unexpected as in agreement with Halden et al. [7] we found that hypoxic serum exhibits and increased activity on systemic arteries. The test on the isolated rabbit auricular artery measures a catecholamine effect [5]. On the umbilical artery which otherwise reacts intensely to noradrenaline and adrenaline, the response was weaker to hypoxic serum, irrespective of its high catecholamine content.

The phenomenon observed may be explained by metabolic effects. It has been shown that if the vascular reaction is performed with different mediators, cGMP increases considerably in the contracted vessel while in the dilated vessel the cAMP content increases [4]. As a result, prostaglandins are produced; of these, only PGE exerts a relaxant effect.

Another possibility is the redox effect of hypoxic serum. The redox potential of the medium exerts a marked effect on the reactivity of isolated organs [14]. At the same time, an increase of cGMP in the isolated umbilical artery could be induced without any oxygen, merely with electron acceptors, i.e. oxidant substances [4]. In connection with these observations we refer to our earlier studies [1] which revealed an enhancement of the reducing activity of hypoxic sera and their antioxidant properties, among others. Together with the present results, these examinations give a deeper pathological interpretation of the justification and mode of action of peritoneal dialysis which we have introduced in the treatment of RDS [2].

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