EFFECT OF THE CONDITIONS OF ANTENATAL DEVELOPMENT ON FUNCTIONAL MATURATION OF RABBIT FETAL SKELETAL MUSCLE

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Exposure of pregnant rabbits to moderate hypoxia, inducing fetal movements, led to sharp acceleration of growth and development both of the fetuses as a whole and of their skeletal muscular system in particular. Excitability of the muscle membrane developed sooner, the mechanical parameters of the muscles were increased, and glycogen synthesis in the liver was intensified by comparison with control fetuses of the same age. Exposure to hyperoxia or powerful stressor stimulation, inducing neurosis and inhibiting the "gestation dominant," reduced the fetal movements. Growth and development of the fetus as a whole and of its skeletal muscular system in particular by a significant degree was accompanied by reduced synthesis of the chief energy-giving material of the muscles (glycogen), both in the skeletal muscles themselves and in the liver.

Experiments in the writers' laboratory [1, 2] have shown that activity of the skeletal muscular system in the antenatal period arises in two ways. Constant tonic activity gives the fetus its specific orthotonic position and performs a circulatory function (the "muscular pump"), supporting the circulation at the required level. The role of the "muscular pump" is particularly important in the other form of activity, which is adaptive in origin in response to a deficiency of food substances and oxygen in the blood, and with the character of an opisthotonic or generalized motor response [1, 3].

The object of this investigation was to study how intensification of the adaptive motor responses or their suppression affects the growth and development of the skeletal muscular system of the fetus.

TABLE 1. Functional Parameters of the Gastrocnemius Muscle of Rabbit Fetuses on the Thirtieth Day of Intrauterine Development

Group of animals	No. of fetuses	No. of fibers tested	Membrane potential (mV)	Action potential (mV)	Duration of action potential (msec)	Amplitude of single isometric contraction (g)	Minimal frequency of continuous contraction (pulses/sec)	Glycogen content (mg %)
Control	10	146	32.4±1.53	30.1±2.3	3.2±0.09	9.3±0.2	15±1.0	1840±10
Hypoxia	10	160	36.7±0.98*	36.8±1.9*	3.0±0.08*	12.1±0.3	19±1.1	1815±25*
Hyperoxia	9	131	30.5±1.52*	27.8±2.2*	3.9±0.1	5.4±0.3	10±0.7	1732±11
Neurosis	8	123	25.9±1.36	20.4±1.8	4.2±0.1	4.0±0.2	7±1.2	1650±27

Note. In the case marked by an asterisk, $P > 0.005$; otherwise $P < 0.01$.

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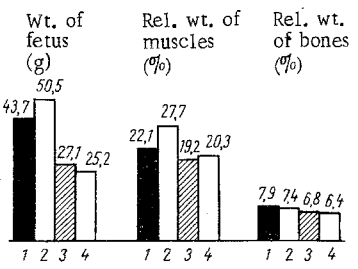

Fig. 1

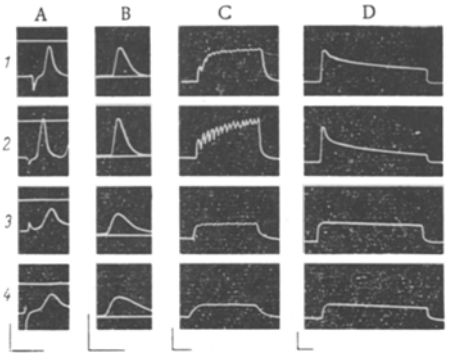

Fig. 2

Fig. 1. Weight parameters of rabbit fetuses on 30th day of intrauterine development under normal conditions and with inhibition of gestation dominant: 1) control; 2) hypoxia; 3) hyperoxia; 4) neurosis.

Fig. 2. Electrical (A) and mechanical (B, C, D) responses of gastrocnemius muscle of rabbit fetuses on thirtieth day of intrauterine development: 1, 2, 3, 4) same groups of animals as in Fig. 1. A) MP and AP. Calibration 20 mV, 10 msec; B) single contraction. Calibration 10 g, 500 msec; C) contraction at 5/sec. Calibration 10 g, 1 sec; D) contraction at 20/sec. Calibration 10 g, 2 min.

EXPERIMENTAL METHOD

In the last third of pregnancy (from the 23rd day), i.e., in the fetal period of antenatal development, pregnant rabbits were exposed to various forms of stress. In the experiments of series I the rabbits were exposed to moderate degrees of hypoxia which, according to earlier observations [1, 2, 7, 8], stimulates adaptive fetal movements. For this purpose the rabbits were exposed daily in a pressure chamber to a pressure equivalent to an altitude of 4 km (12.75% O_2). Exposure lasted 3 h. In series II the rabbits were exposed to hyperoxia in a pressure chamber with an oxygen concentration of 70-80%. Exposure again lasted 3 h. Previous experiments in the writers' laboratory [1, 8] showed that exposure in this way inhibits the adaptive motor responses of the fetus. The same result was obtained in the experiments of series III, in which inhibition of the gestation dominant was induced by the formation of an experimental neurosis for 2 days, on the 22nd and 23rd days of pregnancy, by the method usually adopted in the laboratory [1, 4, 8]: simultaneous electrical stimulation of bare parts of the rabbits' limbs and powerful acoustic stimulation (siren) for 30 min. On the 30th day of intrauterine development the fetuses were taken from the experimental rabbits, including those with a normal pregnancy (control), by caesarian section. In some fetuses the body weight, the total weight of the muscles, and also the absolute and relative weight of the bones were determined. Glycogen in the muscles and liver was determined by the anthrone method [11] and the results were expressed in mg % for fresh tissue. Other fetuses from the same litters were fixed securely by the hind limb in the usual way [6], without anesthesia, and the membrane potential (MP) and action potential (AP) of the muscles fibers of the gastrocnemius muscle were recorded intracellularly by means of glass microelectrodes, filled with 3 M KCl solution, in response to indirect stimulation. The body temperature was maintained at 36.5-36.8°C and the dissected muscle was kept constantly moist with warm physiological saline. To measure the mechanical responses of the muscle under isometric conditions and at different frequencies of stimulation the distal end of the muscle was detached from the bone together with the periosteum and secured to a strain gauge of appropriate sensitivity. The electrcial responses of the muscle were led through a UBP1-02 amplifier and the mechanical responses through a TU-4M amplifier and recorded on a type N-700 loop oscillograph. The experimental results were subjected to statistical analysis and Student's criterion was used to compare the experimental and control measurements.

EXPERIMENTAL RESULTS

The experiments were carried out on 21 fetuses (from 5 rabbits) with normal intrauterine development, 25 fetuses (from four rabbits) developing during exposure to hypoxia, 19 fetuses (from four rabbits) developing in hyperoxia, and 22 fetuses (from five rabbits) developing during stess-induced neurosis.

The weights of the fetuses investigated are given in Fig. 1. They show that exposure to moderate hypoxia led to marked acceleration of body growth, due primarily to growth and development of the skeletal musculature, while the mass of the bones remained unchanged from that of control fetuses of the same age. Exposure to hyperoxia and inhibition of the gestation dominant by stess both led to marked delay in growth of the fetus as a whole and of the skeletal musculature in particular.

Investigation of the functional parameters of the skeletal muscles (Table 1) showed that during hypoxia these parameters develop sooner: a higher level of polarization of the muscle fibers, reversal of the AP appearing for the first time, but still only very slight in degree, and a decrease in the duration of the AP compared with these parameters in the control fetuses. These characteristics reflect an increase in the potential lability of the skeletal muscles of the animals actually during antenatal development. According to Diamond and Miledi [10], tonic activity of the skeletal muscles of the fetus at this age in animals such as rabbits that are born blind is produced by low-frequency and low-amplitude local potentials or local end-plate potentials (EPPs), in consequence of the generalized cholinergic receptor activity of the muscle membrane characteristic of that age. During exposure to hyperoxia and neurosis there was a sharp decrease in MP and a decrease in amplitude of AP. Differences between the electrical responses of the gastrocnemius muscle in fetuses with different conditions of intrauterine development are clearly visible in Fig. 2. For instance, whereas in the fetuses in the experiments of series I the first signs of conversion of the EPP into a spreading AP can be seen, in the experiments of series II and III the muscle EPPs are protracted in character. Comparison of the mechanical characteristics of the gastrocnemius muscles (Fig. 2, Table 1) also shows that the working capacity of the fetal skeletal musculature is increased if their growth is accelerated (hypoxia) but sharply reduced if their growth is delayed (hyperoxia and neurosis). If the potential lability of the skeletal musculature is judged from the minimal frequency of stimulation required to produce a continuous contraction [5], as Table 1 shows, in series II this frequency was increased and came close to the values found during postnatal development. In series II and III it was reduced, indicating a lowered value of the potential lability. During prolonged stimulation at 20/sec for 10 min, no evidence of fatigue (a sudden drop in the strength of mechanical contraction) was observed during isometric contraction. Meanwhile in the fetuses in the experiments of series II and III clear evidence of a drop in mechanical contraction was found after only 2-3 min.

Under the conditions of a normal pregnancy, when natural phsiological hypoxemia occurs [1, 2, 9], the principal source of energy for the working activity of the muscles is anaerobic breakdown of carbohydrates, especially glycogen [3]. For this reason it was decided to determine the glycogen content in the skeletal muscles and liver of the fetuses, the principal glycogen depots. The results showed that the glycogen concentration in the muscles was not significantly changed during intrauterine development after exposure to the various factors (Table 1) although there was a tendency for it to decrease in the experiments of series III. The glycogen concentration in the liver, however, varied considerably depending on the character of intrauterine development. In the fetuses in the experiments of series I, for instance, it was higher (4,600 ± 32 mg %) than in the control (4,020 ± 30 mg %). It fell sharply in the fetuses in the experiments of series II and III (1,865 ± 28 and 1,115 ± 18 mg %, respectively).

The decrease in adaptive motor activity in the fetuses exposed to hyperoxia or to inhibition of the gestation dominant thus leads to delay in the growth and development of the skeletal muscular system, whereas stimulation of adaptive motor responses by hypoxia leads to the acceleration of growth and development of the skeletal muscular system.

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