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Dynamics of the Formation of Rhythmic Activity of the Heart in Fetuses and Newborn Rats

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The development of heart activity and its relationship with respiratory and motor activities were studied in rat fetuses with preserved placental circulation on gestation days 15-20 (E15-20) and in newborn rats (P0). During the studied period, the heart rate in fetuses increased from 175.93±6.10 bpm (E15) to 271.82±5.93 bpm (E20). After birth, the heart rate decreased to 220.94±8.73 bpm. Heart rate variability in the decasecond and near-minute ranges was detected. At E16 stage it is presented by slow regular oscillations lasting for 20-35 sec with an amplitude of 10-45 msec. Comparison of functional activities of the cardiac and somatic motor systems showed that at E16, fluctuations in heart rate are independent of the bouts of motor excitation. During growing, the degree of synchronization of heart rate variability with physical activity increased. E17-18 stage is characterized by short-term episodes of heart rate deceleration associated with motor activity; their duration and amplitude did not depend much on the force of movement. At E19-20, decelerations typical of early gestation terms were replaced by acceleration-type reactions typical for mature organism, which is related to maturation of coordination function of the nervous system. In the heart rhythm, respiratory arrhythmia appears during episodes of rhythmic breathing. Newborn rats demonstrated acceleration episodes; their parameters depend on the force of motor bouts; respiratory arrhythmia was not observed.

Key Words: *rat fetus; heart rate; slow-wave activity; breathing and movement; cross-system interaction*

Despite the fact that heart rate, respiration, and motor activity in mammals appear long before birth, these processes in the fetal period are poorly studied. A paucity of *in vivo* physiological studies during the fetal period [6,10,11] makes it difficult to retrace the genesis of these functions from their emergence to physiological maturity. In addition, experimental results vary in the different animal species. Most prenatal studies are performed on sheep fetuses [8,12,13]. Ontogenetic studies on rats are of special interest, because many physiological functions in altricial animals develop after birth. This allows us to study their organization at the early stages of development.

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Here we studied age-related differences in HR dynamics and the slow-wave (modulating) component of the heart rhythm in rat fetuses and newborn rats. We also studied the basic patterns of changes in heart rhythm depending on activity in the motor and respiratory systems. The results can be of practical interest, because indicators of cardiac rhythm, locomotor and respiratory activities of fetuses are now used in clinical practice for evaluation of their functional state [1,4,7].

MATERIALS AND METHODS

Two experimental series were conducted on fetuses on gestation days 15-20 (E15-20) and on newborn Wistar rats (P0).

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The first series of experiments was carried out on fetuses extracted from the uterus with intact placental circulation. The uterus was exteriorized in female under weak urethane anesthesia (1 g/kg) and epidural anesthesia with lidocaine (20 mg/kg) and immersed in a temperature controlled (38.0±0.5°C) saline bath. ECG, breathing movements and, from the E18, electromyography (EMG) of neck muscles were simultaneously recorded in the fetus. To minimize damage to the fetus, single lead ECG was recorded at E15-18 (active electrode was placed subcutaneously directly over the heart, and the indifferent, into the surrounding saline solution); in E19-20 fetuses, ECG in standard lead II was recorded. Respiratory movements of the fetus were recorded using piezoelectric sensors. Videotaping was conducted throughout the experiment. Introduction of electrodes for EMG recording to E16-17 fetuses caused complete cessation of their movements, therefore motor activity during this period was estimated by signals from piezoelectric sensors and by the analysis of videotapes. ECG and respiration of the female were recorded to control its condition and detect possible impact of its functional state on fetal activity. The total time of the study of each fetus varied from 30 to 120 min. The results were obtained on 130 fetuses from 49 females.

In the second series, experiments were performed on 20 newborn rats within the first 3-4 hours after birth. The animals were placed into a soundproof chamber with constant temperature of 28.0±0.5°C. The extremities were gently fixed on a wax plate with strips of plaster. For ECG recording needle electrodes were introduced under the skin of parietal part of the head and sacral region to minimize the noise associated with muscle activity. Simultaneously, EMG of gastrocnemius muscle and chest excursion were recorded using a piezoelectric sensor.

Signals through L-154 and E14-440 ADC were feed into the computer (L-card, programs Oscilloscope and PowerGraph 3.3) and sampled for 3.5 min to 10 min epoch at a rate of 0.5 or 1 msec per point. Signals were processed using Origin 7.5 software. Cardiac function was evaluated by two parameters: mean HR and changes in *R-R* intervals. The data was processed statistically. The differences were evaluated by Student's *t* test.

RESULTS

At E15 only ECG was recorded, because motor activity (MA) and respiratory activity are absent at this age. There was considerable individual variation in fetal HR from 146 to 209 bpm. The dynamics of the mean values of the studied physiological parameters in presented in Table 1.

MA first appears in 16-day fetuses. Animals are active on average 26% time. At this age, all fetal breathing movements (BM) are single, followed by intervals of 20-40 sec and are invariably accompanied by the general extensor body jerk. At E16, fetal HR increases by 17.5% compared to E15 and varies in a wide range from 160 to 255 bpm. In 60% fetuses, marked periodic changes in *RR*-intervals presented by slow oscillations with a duration of 20-35 msec at amplitude of 10-45 msec were observed; they were most regular when the fetus is motionless (Fig.1, *a*). Similar sinusoidal oscillations of cardiac rhythm with a frequency of 2-5 per minute sometimes occur in

TABLE 1. Dynamics of Cardiac, Respiratory, and Motor Activities in Fetuses of Different Gestational Age and Newborn Rats (*M*±*m*)

Age	Heart rate, bpm	Time of fetal activity, % of total observation time	Mean duration, sec		Number of BM per minute**
			bouts of generalized movement	interval between bouts of generalized motions*	
E15 (*n*=17)	175.93±6.1	-	-	-	-
E16 (*n*=15)	206.78±12.16^{+}	26.01±3.24	17.12±1.76	52.7±7.21	1.54±0.17
E17 (*n*=18)	211.19±9.33	31.69±2.54^{++}	23.421±1.379^{++}	49.262±5.198	3.49±0.34^{++}
E18 (*n*=31)	221.77±6.82	39.14±5.18	36.73±12.03	30.22±2.77^{++}	3.68±0.29
E19 (*n*=21)	254.28±10.05	20.1±7.21	16.62±2.51	48.73±7.92^{+}	2.56±0.23^{++}
20 (*n*=28)	271.82±5.93	8.44±2.01*	9.74±1.03^{+}	-	1.21±0.14^{++}
P0 (*n*=20)	220.94±8.73^{++}	27.71±2.87^{++}	34.75±2.96^{++}	46.85±2.96	-

Note. $^{+}p<0.05$, $^{++}p<0.01$ in comparison with the previous gestational age. *Immobility intervals >3 min are excluded; **number of single BM excluding periods of continuous periodic breathing.

human fetuses and are considered as an indicator of severe fetal pathology [5,9]. During movement, the oscillations persist, but their regularity is disturbed (Fig. 1, *a*). At this age there is no clear relationship between oscillations of cardiac rhythm and bouts of motor activity.

The pattern of MA in 17-day fetuses differed little from this at E16. Heart rate slightly increased. A relationship between HR oscillations and MA bouts appeared (decelerations during movement; Fig. 1, *b*). Transient decelerations not associated with movements were also observed. Decelerations associated with MA

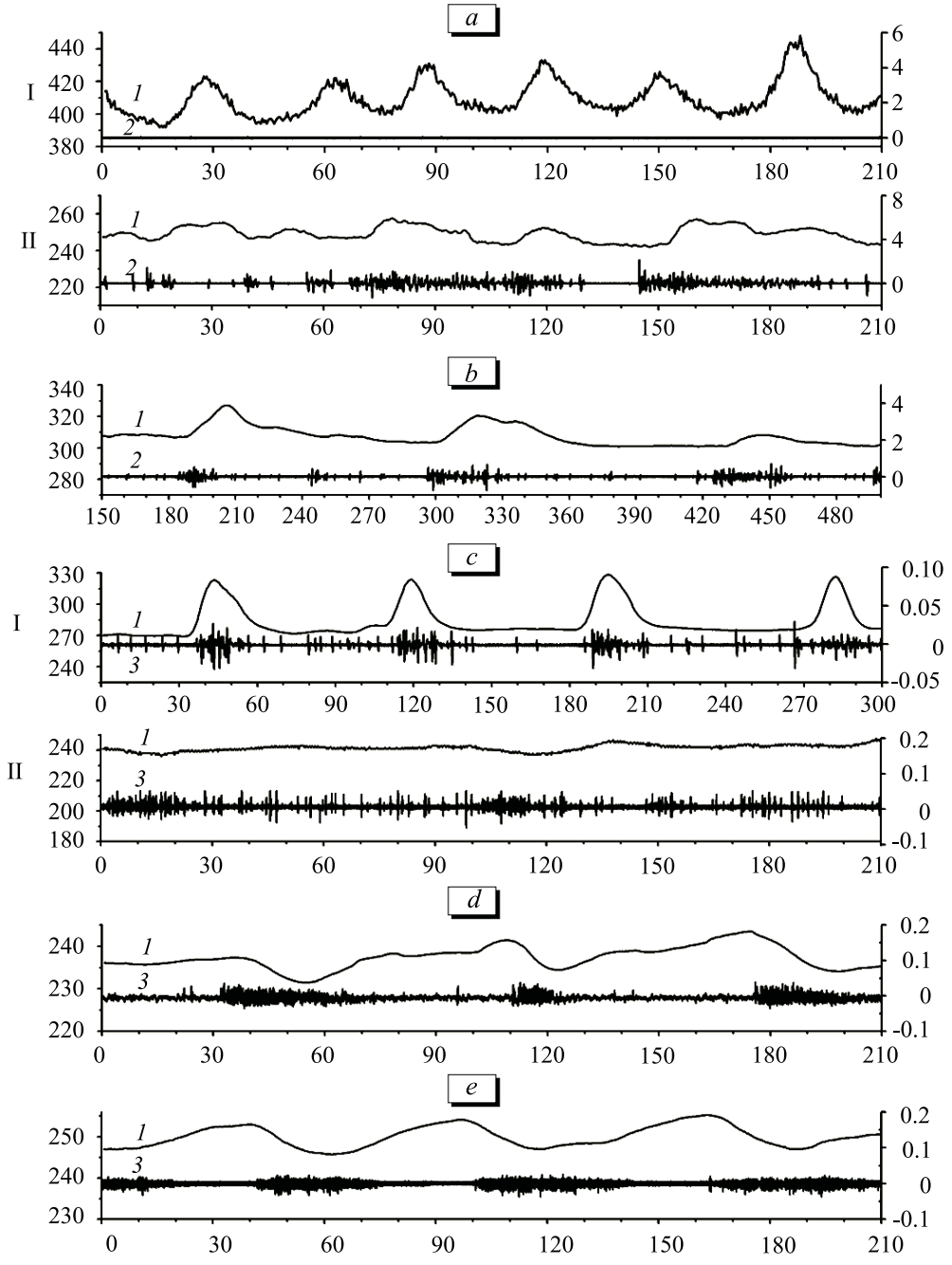

Fig. 1. Slow-wave modulation of heart rate and motor activity in fetuses of different gestational ages and newborn animals. *a*) E16: fluctuations in heart rate in the absence of motion (I) and during bouts of motor activity (II); *b*) E17: transient decelerations during bouts of motor activity; *c*) E18: decelerations during bouts of motor activity (I) and smoothing of the oscillations during continuous motor activity (II); *d*) E19: decelerations alternating with palpitations (accelerations) during generalized motions; *e*) P0: fluctuations in heart rate in the form of accelerations.*1*) periodogram, *2*) piezogram of movements, *3*) EMG of neck muscles. Abscissa: time of observation (sec); left ordinate: amplitude of periodogram of *RR*-intervals (msec); right ordinate: amplitude of piezogram (*a*) and EMG (*b*-*e*) (mV).

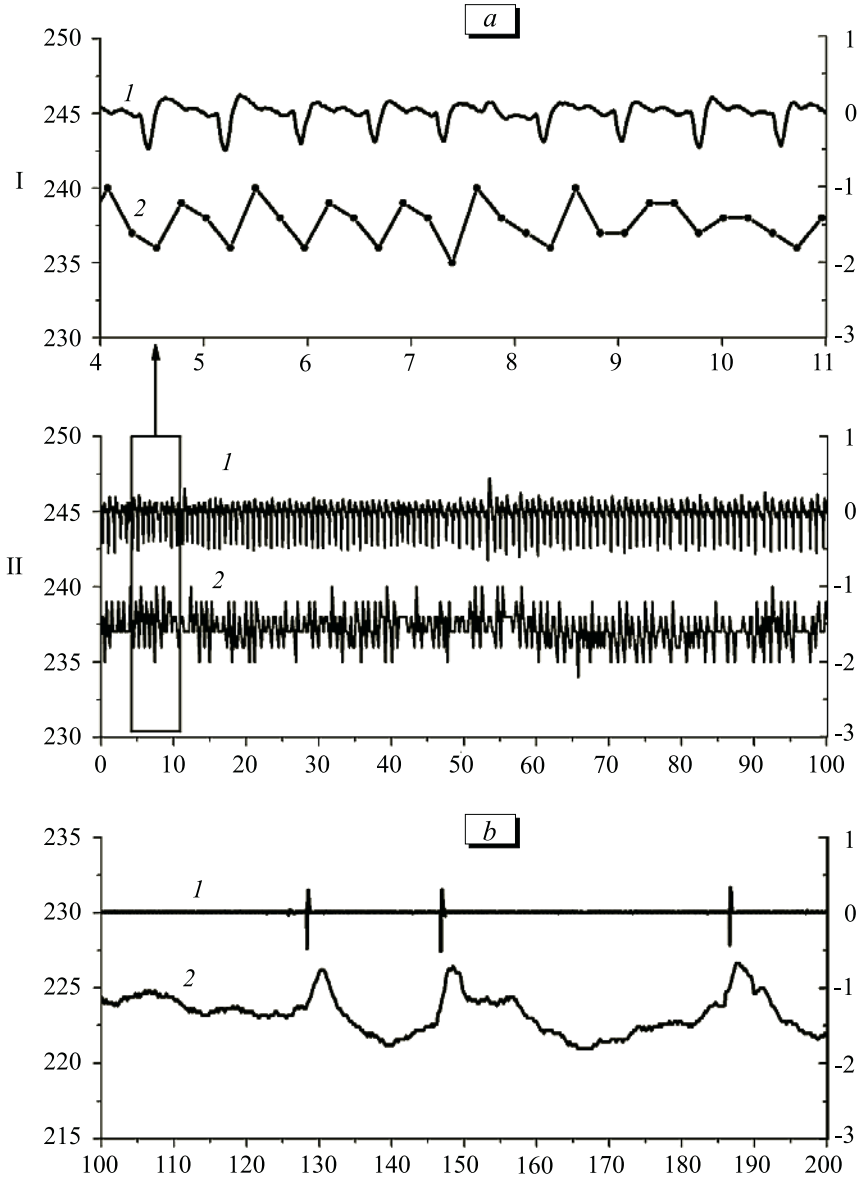

Fig. 2. Piezogram of BM (*1*) and periodogram of *RR*-intervals (*2*) of the fetus (E19). Tendency to synchronization of fast heart rhythm oscillations with BM (*a*); deceleration during single BM (*b*). *a*(I) shows a fragment of the record *a*(II). Abscissa: time of observation (sec); left ordinate: amplitude of periodogram (msec), right ordinate: amplitude of piezogram (mV).

are also characteristic of the human fetus. Taking into account the occurrence of decelerations in anencephaly, is can be concluded that higher CNS structures are not involved in their genesis.

On day 18, fetal MA reached its maximum, the animals were active about 40% time. The mean number of BM increased and a tendency to their grouping appeared (3-10 movements at intervals of 1-5 sec). At E18, heart rate increased by about 5%. In 62% fetuses, cardiointerval oscillations with the wave duration of 20-40 sec and with amplitude of 10-60 msec were recorded. Synchronization of cardiac rhythm oscillations and MA increased. Decelerations precede motor bouts or start simultaneously with it. They are usually fairly uniform and their duration and amplitude weakly depended on the force of movement (Fig. 1, *c*). During transition to continuous MA, slow periodic changes of *RR*-intervals were smoothed (Fig. 1, *c*).

The pattern of MA in E19 fetuses is a transition from E18 to E20. Single BM are rare at E19, but episodes of rhythmic fetal breathing with a duration up to 5 min were observed. At the beginning and end of the episode, the intervals between different BM varied. In the middle of the episode, the rate became constant and reached a maximum value of 0.8-2.0 Hz (40-110 per minute), which is close to the frequency of neonatal breathing. The mean HR at E19 continued to increase. Heart rhythm variability sharply decreased. Pronounced slow periodic fluctuations are recorded in only 19% fetuses. Their amplitude decreased to 2-15 msec and the period increased to 40-60 sec. Single nonperiodic decelerations associated with movements predominated. Episodes of increased HR (accelera-

tions) during generalized motions were first recorded. Three fetuses demonstrated a biphasic change in heart rhythm during the movement: short deceleration at the beginning of the movement was followed by acceleration (Fig. 1, *d*). This kind of interaction is described in the rats at first two postnatal weeks [3]. Transition from decelerations to acceleration-like responses characteristic of mature organism is associated with maturation of the coordination function of the nervous system and is considered as an important functional indicator of human fetus [1,4]. Heart function in E19 and E20 fetuses is affected by their respiratory activity. During episodes of rhythmic breathing, three fetuses showed rapid cardiac rhythm fluctuations comparable with the frequency of BM (Fig. 2, *a*). Indivisual breaths were often accompanied by changes in heart rhythm. It is noteworthy that single BM were accompanied by decelerations (Fig. 2, *b*), while "expirations" during rhythmic breathing were accompanied by accelerations.

By day 20, MA level markedly decreased to 42% of the level opbserved at E19. The number of single BM decreased by more than 2 times. The duration of rhythmic breathing episodes increases to 7 min. At E20, the mean HR increases by 7%. Slow periodic oscillations of *RR*-intervals sharply decreased. Oscillations of heart rhythm persist in the form of weak accelerations with an amplitude up to 10 msec in response to generalized motion and decelerations in response to separate breathing movements.

In newborn rats the level of MA increased again, they were active about a quarter of the observation time. The mean HR considerably decreased in comparison with that at E20. During generalized motions, 85% animals showed oscillations of cardiac rhythm in the form of accelerations. Their duration and amplitude directly depended on the force of motor bout (Fig. 1, *e*); the amplitude varied from 5 to 15 msec. Along with accelerations, 25% animals demonstrated single short-term decelerations not associated with motor activity. Despite the appearance of independent rhythmic breathing in newborn rats, none of the tested animals developed respiratory arrhythmia.

Thus, the first interactions between the studied systems in rats begin to form already during the fetal period. These interactions are realized either via direct influence of one system to another, or due to the existence of external sources of rhythms for these systems. The first mechanism undoubtedly determines high-frequency oscillations of heart rhythm associated with breathing (respiratory arrhythmia). It is also likely to result in the emergence of generalized acceleration-like reactions to movements. The second mechanism can be responsible for slow oscillations in heart rhythm in the form of decelerations. In our previous study on newborn rats, temperature oscillations were documented at the near-minute–near-second ranges coinciding with the development of the rhythmic excitation in several functional systems [2]. It was found that these rhythms reflect the dynamics of metabolic processes, and they are possibly involved in synchronization of rhythmic activity in different structures. This synchronization mechanism apparently works during the fetal period of rat development.

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