PHYSIOLOGY

Reproduction of the Primary Rhythms of Excitation in Cardiac Activity of Neonatal Rat Pups

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It is shown that during activation of the central N-cholinergic systems, the cardiac pacemaker elements in neonatal rat pups are capable of switching between discontinuous rhythmic activity and the generation of periodic excitation, when complexes of cardiac contractions at a rate of 60 min^{-1} alternate with 5-15-sec intervals of rest. This type of activity is close to the primary rhythms of excitation, which are characteristic of the activity of excitatory structures during the early phylo- and ontogenetic stages of development.

Key Words: *ontogenesis; cardiac rhythm; cholinomimetics*

The paramount importance of periodic processes in living matter is now hardly disputed. On the other hand, despite scrupulous study of the cyclic pattern of certain biochemical or physiological process, scientists have paid virtually no attention to the ancient (primary) rhythms of excitation which are characteristic of different excitatory structures at the early phylo- and ontogenetic stages of development [3].

Right from the earliest stages of development, cardiac activity is a discontinuous rhythmic process, and the cholinergic system plays a crucial role in its stabilization and transformation [1,7]. What is not yet clear is whether the ability of the cardiac pacemaker and regulatory mechanisms to change the pattern and parameters of the cardiac rhythm is stringently limited. Is it possible, by changing the conditions of its regulation, to reveal the signs of the primary rhythms of excitation in cardiac activity? We have attempted to answer these questions in this study, drawing on the methods of evolutionary physiology: the ontogenetic method and the method of experimental injuries.

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MATERIALS AND METHODS

The experiments were carried out on Wistar rat pups aged from 5 to 16 days. The cardiac rhythm was analyzed in intact rat pups, recording the electrocardiogram (ECG) (standard lead II) with needle electrodes. Five series of experiments (8-10 animals in a group) were performed, whereby the rat pups were given intraperitoneal injections of a number of cholinomimetics: acetylcholine (500 mg/kg), arecoline (0.01 mg/kg), nicotine (3 mg/kg), cytizine (50 mg/kg), and hydroperoxide, a nonspecific cholinomimetic [6], in a dose of 115 mg/kg, corresponding to 50% of the median lethal dose. The control group of rat pups received

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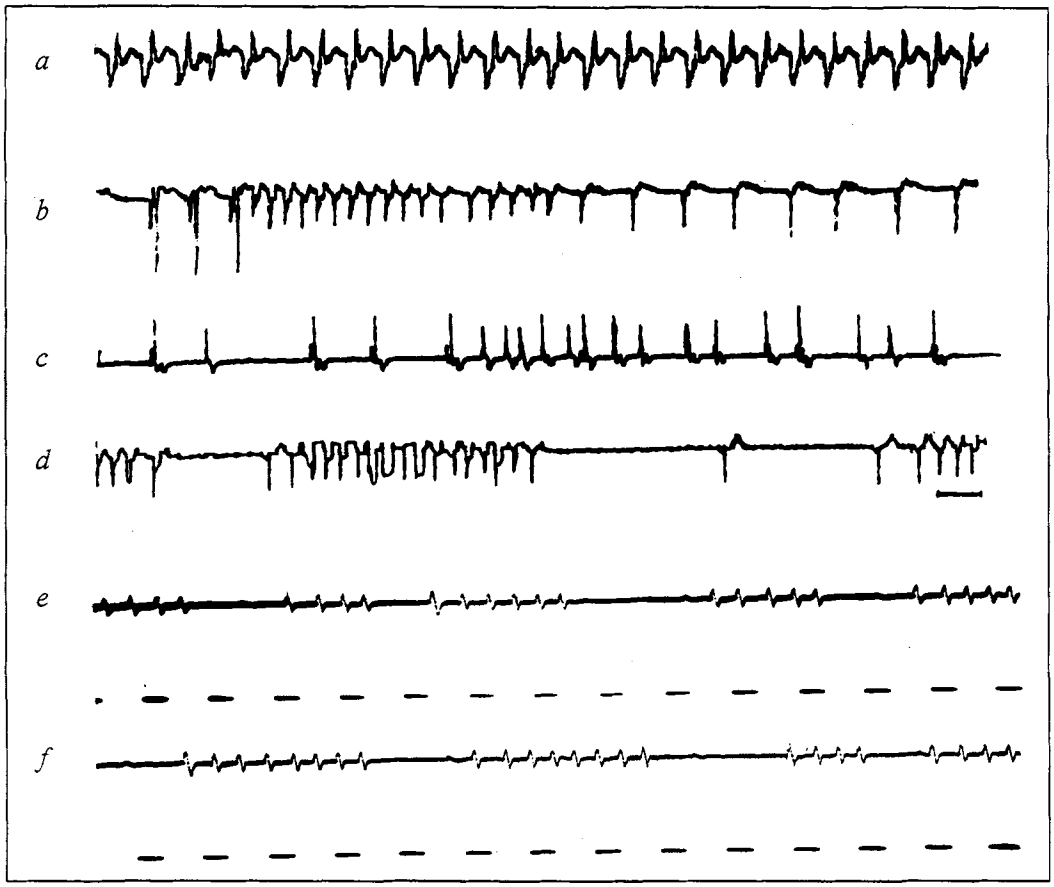

Fig. 1. Mechanocardiograms of 5–7-day rat pups after injection of physiological saline (*a*), acetylcholine (*b*), nicotine (*c*), cytisine (*d*), and hydroperoxide (*e* and *f*: continuous recording) under conditions of pneumothorax. Note the emergence of complexes of rapid contractions (*b* and *c*) against the background of bradycardia and their modulation by the rhythm of periodic excitation (*d*–*f*). Time marks: *a*) 1 sec; *b*–*f*) 2 sec.

physiological saline. The experiment was then conducted in two directions: in some rat pups the ECG was recorded during 45 min after injection of the preparation; the remaining (5-6) animals underwent the following procedure. Five minutes postinjection, the stem of the vagus was bilaterally exposed at the neck under ether anesthesia. A fenestra was excised in the ventral part of the chest, and the pericardium was opened. A VP-102 RFT piezotransducer (Germany) was passed with a micromanipulator to the exposed epicardial surface of the right ventricle, and the mechanocardiogram was recorded on an F-37 computer, photorecording being performed in parallel on an FOR-2. During the investigation, the surface of the heart was irrigated with physiological saline. After pneumothorax and respiratory arrest, cardiac activity was preserved for 30-90 min. Precisely this variant of investigation yielded the best pronounced results. After a stable cardiac rhythm was established, bilateral transection of the vagus was performed, and the mechanocardiogram was recorded till the arrest of cardiac activity.

The qualitative response to the pharmacological preparations was assessed in the study.

RESULTS

In the rat pups used in the study the heart rate (HR) was lower than in adults and varied from 250 to 350 beats per min. Since the results were most informative during pneumothorax, it is this series of experiments which is described below.

During the first few minutes of recording (10-15 min postinjection), the normal sinus rhythm with an HR of about 60 beats/min was preserved in the rat pups (Fig. 1, *a*), gradually slowing to 20-40 beats/min. Later, the responses caused by injections of M- and N-cholinomimetics had different patterns. The central M-cholinomimetic arecoline, as well as physiological saline, did not disturb the regular rhythmic pattern in the control group. A gradually progressive increase of the RR intervals, culminating in cardiac arrest, was observed. Bigeminy was noted in just one case (in a 7-day pup).

Injection of acetylcholine and N-cholinomimetics resulted in the transformation of the sinus rhythm. Two levels of responses could be distinguished depending on their intensity. After injection of acetylcholine or nicotine, the complexes of rapid contractions appeared at intervals of the order of seconds (Fig. 1, *b, c*). Such complexes may appear either regularly or randomly. Injection of cytisine or hydroperoxide resulted in the development of the second-level responses. This manifested itself in the regular emergence of the above-mentioned complexes of contractions (at a rate of about 60 beats per min) with a period of about 8-20 sec, contractions between complexes being virtually absent (Fig. 1, *d-f*), i.e., the rhythm of a higher order (the rhythm of periodic excitation) modulated the rhythm of cardiac contractions. During early ontogenesis, the rhythms of excitation which have the same temporal parameters are characteristic of the states of motor excitation [2,3]. The test preparations, with respect to the intensity of responses caused by them, were ranked as follows: hydroperoxide > cytisine > nicotine > acetylcholine. It should be mentioned that in the groups of pups in which the ECG was recorded before and after injection of the preparations, first-level responses, i.e., the complexes of rapid contractions, could be observed; however they manifested themselves only against the background of a regular sinus rhythm.

Some scientists have established [3,5,8] that a specific (primary or endogenous) rhythmic pattern of excitation is characteristic of the functioning of different excitatory structures at the early stages of phylo- and ontogenetic development. This is most evident in the structures of the immature somatic nervous system. In phylogenetically different representatives the major rhythms may be subdivided into 3 groups with periods of some 1, 10-15, and 60 sec [2,3]. During ontogenesis, the capacity for endogenous excitation is suppressed in the cell. This brings us directly to our experiments. It has been shown previously that under conditions of alteration the latent self-maintenance of a rhythmic pattern becomes active [3,4]. Thus, under certain unfavorable conditions the regulatory mechanisms established at the early prenatal stages are disturbed, and this allows the primary rhythms of excitation to manifest themselves in structures which do not exhibit them under normal conditions. In this context, the results of research into the effect of oxygen at a high partial pressure were especially demonstrative [4]. Our findings concerning the effect of hydroperoxide, which causes several effects all at once (activation of the cholinergic systems, blood oxygenation, which protects the myocardium from ischemia, and, finally, the toxic effect of oxygen, resulting in disturbance of the mechanisms of cardiac innervation) are noteworthy in this connection. It should be mentioned that the responses described in our study are characteristic of the early stage of postnatal ontogenesis and do not manifest themselves in adult animals [6].

The order of intensity of the effects of several preparations, which was established in our study allows us to speculate on the mechanisms of cardiac rhythm transformation. Evidently, in this case the crucial role is played by the afferent activation of the central N-cholinergic structures, which in turn potentiates excitation of the elements responsible for the generation of the primary rhythms. The more pronounced the alteration, the stronger the activation of the cholinoreceptors, and the easier the liberation of ancient endogenous rhythms. As follows from our unpublished data, it is precisely the central N-cholinergic systems which provide for the realization of spontaneous periodic motor excitation in neonatal rat pups. It was also found that such an effect is mediated by the descending catecholaminergic systems, and the cholinergic systems exert tonic modulatory effects on the activity of their central neurons.

Hence, our findings allow for the conclusion that, first, the cardiac pacemaker elements are able to reproduce, under certain conditions, the endogenous rhythms of periodic excitation characteristic of the activity of the immature nervous system; and, second, the central N-cholinergic systems play an important role in maintaining the generation of the primary rhythms.

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