DYNAMICS OF ACETYLCHOLINESTERASE AND BUTYRYLCHOLINESTERASE ACTIVITY IN THE HEART TISSUES OF DOGS DURING POSTNATAL DEVELOPMENT

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Activity of acetylcholinesterase (ACE) and butyrylcholinesterase (BCE) was studied in the region of the sino-atrial node, the atrio-ventricular node, and the contractile myocardium in puppies aged from 1 day to 2.5 months and in adult dogs. The results showed that ACE and BCE activity in all parts of the heart decreases with age. This decrease correlates with the appearance and strengthening of vagal control over cardiac activity and the slowing of the heart rate developing in dogs during postnatal development. At all age periods a decrease in ACE and BCE activity is observed from the sino-atrial node to the apex of the heart; this is evidently linked with the gradient of automatic activity of the heart and the distribution of acetylcholine in the heart. At all age periods BCE activity predominates over ACE activity but its decrease with age was more marked.

Constant tonic effects of the center of vagal innervation on the heart are absent in puppies from the ages of 1 to 16 days; cardiac activity is regulated by the sympathetic innervation, which maintains a high rate of cardiac contraction in a resting state. Meanwhile the peripheral cholinergic system is already represented in puppies of this age, so that vagal bradycardia can develop in response to acute hypoxia, increased intracranial pressure, and the action of morphine. Constant vagal influences arise after 16-18 days of life and strengthen with age, lowering the heart rate and giving rise to peripheral cholinergic effects [2-4, 6, 9-11].

The role of the acetylcholine–acetylcholinesterase system in the heart in the changes taking place in cardiac activity during ontogeny was investigated. Since the cholinergic system in the heart is represented not only by acetylcholinesterase (ACE), but also by butyrylcholinesterase (BCE), the activity and distribution of both enzymes were investigated in the nodes of the heart and the myocardial tissue.

EXPERIMENTAL METHOD

Experiments were carried out on 25 puppies aged 1-2, 10-12, and 25-30 days and 1.5-2.5 months and on six adult dogs. The animals were killed by electrocution. Activity of ACE and BCE was determined in heart tissue extracts. In each experiment samples were taken from three parts of the heart: the regions of the sino-atrial node, the atrio-ventricular node, and the contractile myocardium of the left ventricle (apex of the heart). Acetylcholine chloride and butyrylcholine iodide were used as the substrates for determination of ACE and BCE activity.

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TABLE 1. Activity of ACE and BCE (in μmoles/g tissue/min) in Dogs' Heart during Postnatal Development (M ± m)

Group of animals	Age of animals	No. of animals in group	Region of sino-atrial node		Region of atrio-ventricular node		Contractile myocardium of left ventricle	
			ACE	BCE	ACE	BCE	ACE	BCE
1	1—2 days	5	1,92 ± 0,10	4,30 ± 0,15	1,21 ± 0,05	3,10 ± 0,06	0,92 ± 0,03	2,30 ± 0,06
2	10—12 "	6	1,63 ± 0,04	2,41 ± 0,05	1,53 ± 0,13*	1,60 ± 0,05	0,51 ± 0,09	0,95 ± 0,03
3	25—30 "	5	1,28 ± 0,08	1,96 ± 0,20*	1,18 ± 0,10*	1,28 ± 0,07	0,69 ± 0,02*	0,79 ± 0,06*
4	1½—2½ months	9	0,93 ± 0,07	1,00 ± 0,06	0,55 ± 0,07	0,75 ± 0,05	0,41 ± 0,01	0,64 ± 0,06*
5	Adult	6	0,68 ± 0,07	0,83 ± 0,07	0,37 ± 0,06*	0,58 ± 0,06	0,26 ± 0,06	0,35 ± 0,08

*In these cases differences between groups 1 and 2, 2 and 3, 3 and 4, and 4 and 5 are not significant ($P > 0.05$).

Note: Differences between groups 1 and 5 significant in all cases ($P < 0.001$).

EXPERIMENTAL RESULTS AND DISCUSSION

The results (Table 1) show that ACE activity of the cardiac pacemaker was highest during the first days of life. The possibility of rapid hydrolysis of acetylcholine maintains a high frequency of automatic excitation at rest. The fact that the ACE activity is almost three times higher than in the adult can also explain the character of the episodic cholinergic effects in the heart in young puppies: the higher threshold of stimulation of the peripheral segment of the vagus nerve, the less marked vagal inhibition, high threshold doses of acetylcholine and eserine evoking bradycardia [3, 11], and the absence of cholinomimetic and cholinolytic phases after injection of atropine [1]. Histochemical and physiological investigations [7, 15] have also shown that the acetylcholine—acetylcholinesterase system differs at an early age from that in adult individuals.

After the first antigravity reactions had appeared (from the 10th-12th day of life with the forelimbs and from the 18th-20th day from the hind limbs), when the first signs of vagal tone appeared, ACE activity gradually decreased in the region of the sino-atrial node. The lowest ACE activity in the region of the pacemaker (and also in the region of the atrio-ventricular node and the contractile myocardium of the left ventricle) was observed in adult dogs, when tonic excitation reaches its highest level in the centers of vagal innervation of the heart. According to Webb [17], the sensitivity of the atria of adult rabbits to vagal impulses and to acetylcholine depends on the ACE activity in them.

It can be concluded from the results showing the dynamics of ACE activity with age that the decrease in the heart rate in dogs at rest is not attributable entirely to the appearance and strengthening of vagal effects, but also to the considerable decrease in ACE activity in the nodes of the heart, especially the sino-atrial node. This conclusion is confirmed by the increase in the acetylcholine content in the dog's heart (especially in the region of the sino-atrial node) with age [15]. Arshavskii [3-5] explains the slowing of the heart under the influence of the developing vagal tone as the result of depolarization of the sino-atrial node by acetylcholine. The frequency of excitation arising automatically in that node is thereby reduced. Depolarization developing in the sino-atrial node is combined with hyperpolarization in the ventricular myocardium.

The results of the present investigation also show that, irrespective of age, activity of both ACE and BCE in dogs is highest in the region of the sino-atrial node, rather lower in the region of the atrio-ventricular node, and lowest in the contractile myocardium of the left ventricle. These results confirm observations made by other workers [1]. The decrease in cholinesterase activity from the pacemaker to the apex of the heart is probably connected with the distribution of acetylcholine [14] and the gradient of automatic activity of the heart, which is established at an early age.

At all age periods BCE activity exceeds ACE activity. This difference is particularly marked at early age periods. The predominance of BCE activity in the nervous ganglia of the heart of newborn puppies has also been observed histologically [7].

The functional role of BCE has not yet been explained. Some workers [12] consider that it hydrolyzes the excess of acetylcholine if its synthesis is increased. Others [13] ascribe it a protective role as an in-

activator of butyrylcoenzyme A, a toxic product of fatty acid metabolism participating in acetylcholine synthesis.

The present observations show that BCE activity decreases more sharply with age in dogs (by 75-81%) than ACE activity (by 64-72%); the differences in activity of the two enzymes at the same age thereby gradually diminish. This does not mean that the high BCE activity at an early age plays the role of a reserve, for its marked decrease with age is accompanied by an increase in the acetylcholine concentration [15].

LITERATURE CITED

1. É. I. Arshavskaya and S. I. Enikeeva, in: Problems in Age Physiology and Pathophysiology of the Cardiovascular System (Proceedings of a Symposium) [in Russian], Moscow (1966), p. 21.
2. I. A. Arshavskii, Physiology of the Circulation in the Intrauterine Period [in Russian], Moscow (1960).
3. I. A. Arshavskii, Outlines of Age Physiology [in Russian], Moscow (1967).
4. I. A. Arshavskii, Vestn. Akad. Med. Nauk SSSR, No. 2, 43 (1968).
5. I. A. Arshavskii, Uchen. Zapiski Leningrad Univ. Seriya Biol. Nauk, No. 22, 118 (1950).
6. S. I. Enikeeva, Fiziol. Zh. SSSR, No. 2, 227 (1955).
7. E. M. Krokhina, Functional Morphology and Histochemistry of the Efferent Innervation of the Heart, Doctoral Dissertation, Moscow (1970).
8. A. A. Pokrovskii, in: Textbook of the Study of Nutrition and Health of the Population [in Russian], Moscow (1966), p. 144.
9. V. D. Rozanova, in: Problems in the Physiology and Pathology of the Human and Animal Central Nervous System in Ontogeny [in Russian], Moscow (1961), p. 154.
10. V. D. Rozanova, Fiziol. Zh. SSSR, No. 9, 1098 (1964).
11. V. D. Rozanova, Outlines of Experimental Age Pharmacology [in Russian], Leningrad (1968).
12. W. Antropol, S. Glaubach, et al., Proc. Soc. Exp. Biol. (New York), 42, 280 (1959).
13. N. J. Cliterow, M. Mitchard, and N. S. Harper, Nature, 199, 1000 (1963).
14. J. Vlk and S. Tucek, Physiol. Bohemoslov., 10, 65 (1961).
15. J. Vlk and S. Tucek, Physiol. Bohemoslov., 11, 53 (1962).
16. J. Vlk and S. Tucek, Physiol. Bohemoslov., 11, 46 (1962).
17. J. L. Webb, Brit. J. Pharmacol., 5, 335 (1950).