THE EFFECT OF ARECOLINE ON THE CARDIAC ACTIVITY OF THE CHICK OF THE CHICK EMBRYO AT VARIOUS STAGES OF ITS DEVELOPMENT

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The literature contains many papers concerned with the question of the effect of vagotropic substances on the heart of the chick embryo. Many authors, using various isolated-heart methods, have ascertained the periods of embryonal development at which the effect of these substances on cardiac activity is apparent and compared them with the presence or absence of nerve elements in the embryonal heart. The results obtained are somewhat inconsistent. Some authors observed the effect produced by the action of vagotropic substances during the pre-innervated stage of cardiac development [12, 13, 16, 18], while others believe that the effect is not apparent until nerve elements have appeared in the heart [11, 14, 17].

In our opinion, the study of this question should be based on the assumption that specific cholinergic structures which participate directly in the realization of the vagus effect are present in the myocardium [1] rather than on the fact of the development of the nerve elements.

Having taken this position, we decided to ascertain how arecoline affects the heart of the chick embryo at various stages of its development as a step towards understanding the problem of the development and manifestation of the inhibitory process, using as the model the cardiac activity of an intact embryo and taking measures for maximum viability of the latter.

METHODS

In the work, we recored the ECG of the chick embryo, providing for its maximum viability [5]. To this end, the egg containing the embryo was placed in a heated chamber; the egg and the embryonal membranes were opened, and the bio-electric potentials of the heart were led off by means of electrodes. After the background frequency of the embryonal heart beat was recorded, arecoline was injected into the thigh in a dose of 100 γ. The frequency of the heart beat was then recorded one, three, five and ten minutes after the arecoline injection. The embryo was then injected with 10γ atropine, after which the frequency of the cardiac contractions was again recorded three, five and ten minutes after the injection. The doses of arecoline and atropine used for each stage of embryonal development were the same to permit comparison of the results obtained. Seventy-three embryos at stages ranging from the 8th to 19th day of development were used in the experiments.

RESULTS

The data obtained showed that arecoline in the dose specified does not exert its characteristic effect on the cardiac activity of chick embryos at early stages of development. We observed no pronounced changes of any kind in the frequency of the cardiac contractions after the administration of the vagotropic substance on the

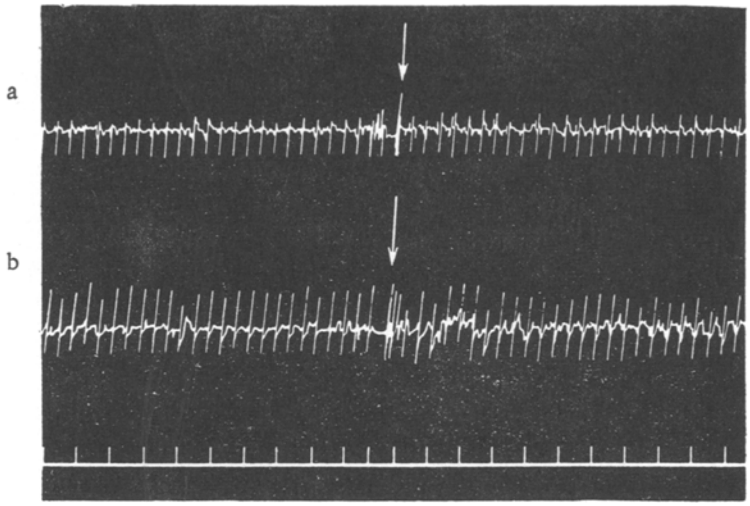

Fig. 1. Absence of change in the frequency of the cardiac contractions after the administration of arecoline to chick embryos on the eighth (a) and ninth day of development (b). Time shown in seconds; arrow represents the arecoline injection. Recorded one minute after the arecoline injection.

8th-9th days of development (Fig. 1). On the eighth day, the injection of atropine on a background of the action of arecoline had no apparent effect either. On the ninth day, a few cases showed some increase in the frequency of the heart beat after the atropine injection.

On the tenth day of development, we observed a more or less expressed decrease in the frequency of the heart beat in several cases the first and third minutes after the arecoline injection, averaging 10-20% of the original level. Five minutes after the arecoline injection, the frequency of the cardiac contractions had usually returned to the original background level.

A peculiar type of cardiac reaction, in the form of alternate decrease and increase in the frequency of the heart beat during the action of arecoline, was observed in a few embryos on the tenth day of development. The unstable, oscillating nature of these changes in the frequency of the cardiac contractions would seem to indicate that the tenth day of development is the first day when the effect of the action exerted by the vagotropic substance is apparent.

After the tenth day of development, this effect became stable, increasing in degree as the embryo grew older. On the 11th day, the decrease in the frequency of the cardiac contractions following the arecoline injection averaged 25-40% of the background frequency, and on the 15th-19th day, this decrease reached an average of 50-60% (Figs. 2 and 3).

The decrease in the frequency of the heart beat was often attended by arrhythmia. The effect of the arecoline injection was usually apparent by the end of the first and beginning of the second minute. Cardiac arrest occurred in a few embryos, but was dispelled by the atropine injection. A somewhat unusual occurrence, difficult to account for, was observed with the repeated administration of arecoline to the same embryo. Although the first injection caused the frequency of the heart beat to decrease, returning to normal by the end of the tenth minute, subsequent injections of arecoline had no effect, even when the substance was injected in a dose of 500 γ.

Atropine, administered to the chick embryos during the decrease in the frequency of the heart beat caused by the arecoline injection, restored the heart rate to its original level; often, we even observed some increase in the frequency of the heart beat over the background level. The atropine injection usually dispelled any arrhythmia which had developed after the arecoline injection.

Visual observation of the embryo's behavior showed a considerable increase in the motor activity of the embryo after the arecoline injection. The movements were irregular and disorganized in nature, alternating with

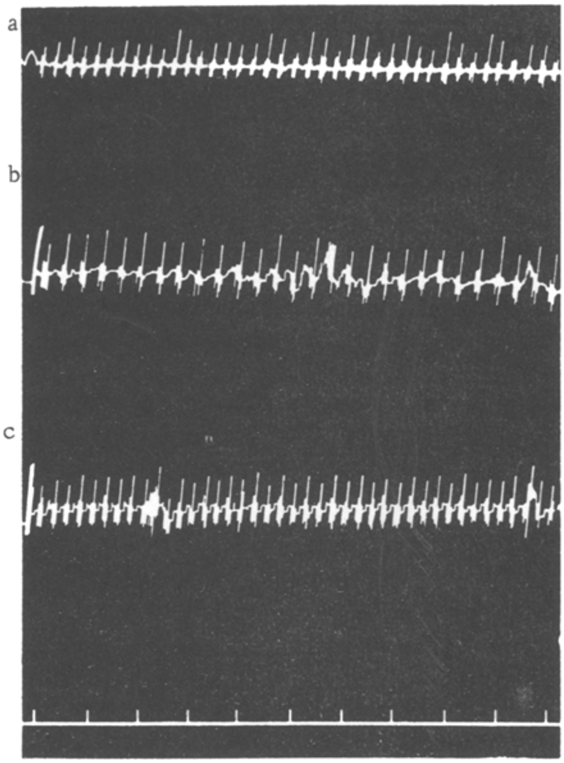

Fig. 2. Change in the frequency of the cardiac contractions effected by arecoline in a chick embryo on the 13th day of development. a) Background frequency of heart beat; b) decrease in frequency of heart beat one minute after arecoline injection; c) restoration of frequency of heart beat three minutes minutes after atropine injection. Time shown in seconds.

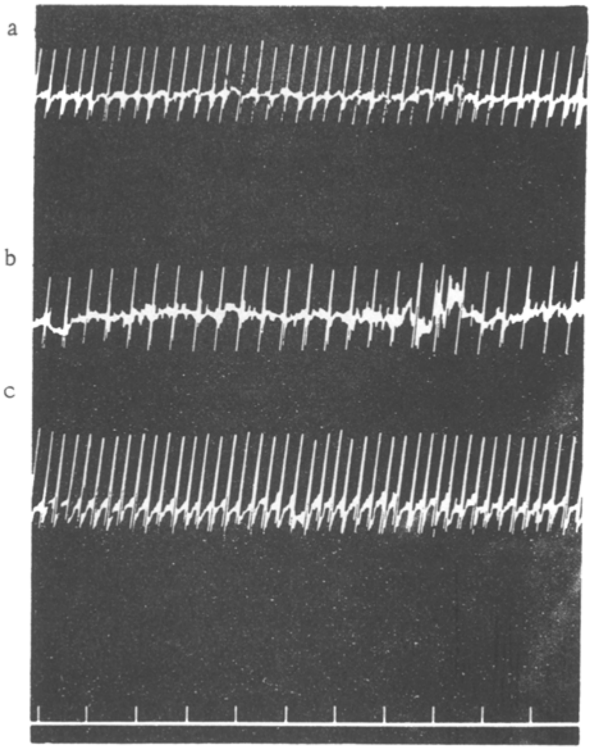

Fig. 3. Change in the frequency of the cardiac contractions effected by arecoline in a chick embryo on the 19th day of development. a) Background frequency of heart beat; b) decrease in heart beat frequency one minute after arecoline injection; c) restoration of original heart beat frequency three minutes after administration of atropine. Time shown in seconds.

short resting periods. Stimulation of the embryo's respiratory movements was observed in several cases after the injection of arecoline on the 19th day.

Several variants of control experiments were conducted in order to establish the exact focus of the action of of arecoline: 1) injection of distilled water instead of arecoline (which caused no change in the rate of the heart beat); 2) injection of arecoline on a background of the action of atropine (arecoline did not exert its characteristic effect on cardiac activity in these experiments). Since atropine, which blocks cholinergic systems, prevented the manifestation of the effect of arecoline, the control experiments confirmed the fact that arecoline is a vagotropic substance and acts on specific cholinergic systems which directly participate in the realization of the inhibitory reaction of the heart. On this basis, the manifestation of arecoline's effect, and therefore, the manifestation of the inhibitory effect of the heart, must be associated with maturation of the analogous physiologic mechanisms or cholinergic structures.

The data obtained show that the cholinergic structures are not functionally in evidence during the initial period of embryogenesis (8th-9th day). Their functional manifestation begins at a definite stage of embryonal development (10th day) but is not immediately complete. In many cases, the inhibitory reaction of the heart was not yet in evidence on the tenth day of development, although it was already clearly apparent in other cases. The oscillating nature of the reaction to arecoline observed in some embryos – periods of decreased heart beat frequency alternating with periods of increased frequency – indicated incomplete functional maturity of the structures producing inhibition of cardiac activity. On subsequent days of embryonal development, the inhibitory reaction of the heart was clearly apparent and stable, increasing with the development of the embryo. It was interesting to observe this characteristic development of cardiac inhibition, which showed the secondary nature of the formation of the inhibitory reaction and the division between the development and manifestation of stimulating and inhibitory influences.

Adrenalin, which stimulates excitation in the substrate, manifests its effect on the heart at the stage of its development observed during the primitive epithelial tube stage [7], but, as our experiments have shown, the capacity for inhibitory reaction is developed much later. Some of the literary data also testify to the later manifestation of the inhibitory reaction of the embryonal heart [2, 5].

This hypothesis is further corroberated in the work of N. A. Itina [6]; after conducting a phylogenic and ontogenic study of the properties of the muscls of various organs, this author concluded that the inhibitory effect of the nervous system on the heart has, in the course of the phylogenic animal development become a secondary phenomenon.

The theory that there is some division between the inhibitory and stimulating processes has been expressed before by I. P. Pavlov (1934). This theory was then developed by L. A. Orbeli [8] and D. A. Biryukov [3], who asserted that the nature of the evolutionary development of nervous activity has ended in the gradual and separate formation of the inhibitory function.

In discussing the question of the division between the development and manifestation of the inhibitory reaction, we have no wish to analogize the inhibitory processes in the central nervous system and in the heart. But posing the question of the times of development of the inhibitory mechanism in the myocardium is commensurate, since excitation and inhibition are functions not only of the central nervous system, but also of highly specialized structures in general [3].

The inhibition effect was first discovered on the model of cardiac activity (Weber, 1845), and discovery of the sequence of development of excitation and inhibition in the heart during embryogenesis can help advance our knowledge of how the interrelations of the principal nervous processes are formed.

SUMMARY

Arecolin which acts on the choline-reactive systems and simulates vagal effect, does not exert typical action on the cardiac activity of chick's embryo 8-9 days old. The tenth day is the first in which arecolin influences cardiac rate. Beginning with the 11th day, administration of arecoline produces constant effect, the magnitude of which grows with the age of the embryo.

Based on the above data the author concludes that the inhibitory reaction of the heart appears and manifests itself at a much later date, and is formed gradually.

LITERATURE CITED

1. S. V. Anichkov, Questions of the Pharmacology of the Autonomic Nervous System [in Russian] (Moscow-Leningrad, 1952) p. 5.
2. I. A. Arshavskii, Transactions of the Seventh All-Union Congress of Physiologists, Biochemists and Pharmacologists [in Russian] (Moscow, 1949) p. 351.
3. D. A. Biryukov, in: Problems of the Comparative Physiology and Pathology of Nervous Activity [in Russian] (Leningrad, 1958) p. 5.
4. O. V. Bogdanov, Fiziol. Zhur. SSSR, 10, 1281 (1959).
5. S. I. Enikeeva and S. M. Shtamler, Byull. Éksp. Biol. Med., 7, 10 (1954).
6. N. A. Itina, in: The Evolution of the Functions of the Nervous System [in Russian] (Leningrad, 1958) p. 288.
7. T. B. Kiseleva, Abstracts of the Proceedings at the Scientific Conference of the Kazan' Institute of Post-Graduate Medicine [in Russian] (1954) p. 8.
8. L. A. Orbeli, The Development of I. P. Pavlov's Legacy in the Field of Higher Nervous Activity [in Russian] (Moscow, 1950).
9. I. P. Pavlov, Pavlovian Media [in Russian] (Moscow-Leningrad, 1949) Vol. 2, p. 364.
10. V. A. Shidlovskii, Transactions of the Seventh All-Union Congress of Physiologists, Biochemists and Pharmacologists [in Russian] (Moscow, 1949) p. 351.
11. P. B. Armstrong, J. Physiol. 84, 20 (1935).

12. W. C. Cullis, and C. L. T. Lucas, J. Physiol., 86, 53 (1936).
13. E. Fingl, L. A. Woodbury, and H. H. Hecht, J. Pharmacol. Exp. Therap. 104, 103 (1952).
14. Fujii and Michio, cited according to Ber. ges. Physiol. w. exper. Pharmakol., 41, 281 (1927).
15. E. K. Hall, J. Cell. Compt. Physiol. 49, 187 (1957).
16. C. Markowitz, Am. J. Physiol. 97, 271 (1931).
17. M. Nordmann and A. Rüther, Arch. exper. Zellforsch. 11, 315 (1931).
18. F. Plattner, and Ch. L. Hou, Arch ges. Physiol. 228, 281 (1931).