

13586

Cardiac Inhibition of a Cladoceran and the Action of Acetylcholine and Physostigmine.

VASIL OBRESHKOVE.

From Bard College, Columbia University.

It has been held by a group of workers that in physiological processes where nervous impulses are involved and where acetylcholine is believed to act as a transmitter of nervous impulses, physostigmine protects the acetylcholine from the destructive action of choline-esterase. The literature on this subject is fully reviewed by Fraser.¹ For demonstrating the action of these drugs on the heart of a number of crustaceans^{2, 3} and on the heart of other invertebrates,^{4, 5} perfusion methods have been utilized in bathing the hearts with the chemicals employed. This has made it difficult (by admission of the workers) to obtain data on the separate and combined action of acetylcholine and physostigmine, particularly when considerable time was spent in the dissection and in the preparation of kymographic recordings. It was found in the experimental results recorded here that if the intestine of *Daphnia magna* is touched with a fine glass needle at the bend of the digestive tube where the stomach enters the intestine, the heart immediately ceases beating and the posterior region of the digestive tube commences to exhibit powerful peristaltic waves. The heart in this respect behaves like the inhibition produced after electrical excitation of the vagus nerves in vertebrates. After a certain period, depending on the strength of the mechanical stimulus employed, both the heart and the digestive tube renew their normal activities. The heart comes to rest in the systolic phase of the cardiac cycle and for a period after the escape from inhibition it exhibits high tonicity. Complete cardiac inhibition can also be produced by touching the heart at its posterior dorsal surface. Since the animals utilized in this work were less than 3 mm in body length, and they were transparent, the changes produced can be readily observed under the microscope. In the absence of other data it is assumed that the region stimulated or perhaps other regions in the immediate vicinity, contain nervous elements which conduct inhibitory impulses leading to the arrest of the heart.

¹ Fraser, F. R., *Brit. Med. J.*, 1938, **1**, 1293.

² Welsh, J. H., *J. Exp. Biol.*, 1938, **16**, 198.

³ Welsh, J. H., *Physiol. Zool.*, 1939, **12**, 231.

⁴ Hamilton, H. L., *J. Cell. and Comp. Physiol.*, 1939, **13**, 91.

⁵ Prosser, C. L., *Biol. Bull.*, 1940, **78**, 92.

Methods. The method employed in this work in producing complete cardiac inhibition in *Daphnia magna* enabled us to make a study of the effects of acetylcholine and physostigmine on the recovery of the cardiac activity of the animal after escape from inhibition. In order that there may be no doubt as to the specific action of the drugs employed, only animals which showed very slow cardiac recovery after the arrest of the heart were chosen for the treatment with chemical substances and for the analysis presented here. The animals were subjected to experimentation soon after the release of the first clutch of young. The method of rearing cladocera in the laboratory and the methods employed in the selection of animals which were in the same stage of development have been described elsewhere.⁶ The technic employed in administering acetylcholine and physostigmine was the same as that employed for the study of the action of these drugs on the intestine of *Daphnia magna*.⁷ The animals were subjected to experimentation separately; a single individual in each case was transferred to a micro culture slide for the treatment with specific chemical substance employed and for observations under the microscope. The solutions were made daily and the acetylcholine was adjusted in each case to pH 5.7. The rate of the heart contractions per minute was recorded by dots on a piece of paper. This method was found accurate for counting heart contractions of about and below 150 per minute but above that it was found more difficult to synchronize the recording of dots with the heart contractions. The error in counting extremely rapid heart beats is not of importance, for the more rapid cardiac activity simply furnishes more unquestionable evidence of the action of the drugs in inducing the specific changes.

Results. Table I presents the results obtained when the heart of *Daphnia magna* was arrested by producing inhibitory impulses with a mechanical stimulus applied over the intestine and then treating the animal with acetylcholine. In this table are given the "normal rate" of the heart beat, the period of heart arrest in each case, the frequency of beat for a period of 2 minutes after the escape from cardiac inhibition as well as after the addition of acetylcholine 10^{-7} . In each case, without a single exception, the heart after the escape from inhibition and before the treatment with acetylcholine showed grouped beats which were feeble in character and irregular in amplitude. This condition persisted for several minutes. After the addition of acetylcholine, however, there was observed a sudden increase in the

⁶ Obreshkove, V., *Physiol. Zool.*, 1930, **3**, 271.

⁷ Obreshkove, V., *Biol. Bull.*, 1941, **81**, 105.

frequency of the heart beat and the cardiac activity became immediately more powerful and regularly rhythmic in character. The drug became effective in each case in less than 20 seconds.

The period of heart arrest is shown in these experiments to vary from 2.5 to 20.7 minutes (column 2). The rate of heart beat during the first minute after escape from inhibition and before the administration of acetylcholine was found to be on the average 57 beats per minute and only slight change in this rate was observed during the following 60 seconds (column 3) and in the course of several minutes later. In the first few seconds after the addition of acetylcholine, however, the rate became rapid (column 4) and in some cases the frequency of beats during the first minute equaled and exceeded the normal rate. Similar observations were recorded with acetylcholine

TABLE I.
Action of Acetylcholine on Recovery of Heart of *Daphnia magna* (First Brood Mothers) from Cardiac Inhibition.
Rate of heart beat expressed in beats per minute.

Normal heart rate	Period of heart arrest min.	Heart beat after recovery		Heart beat in Acetylcholine 10 ⁻⁷	
		Rate	Min. after recovery	Rate	Min. after recovery
226	17.2	18	1	122	1
		36	2	144	2
234	2.3	24	1	246	1
		30	2	244	2
238	20.7	36	1	98	1
		44	2	104	2
250	16.6	72	1	170	1
		130	2	180	2
260	19.3	80	1	122	1
		80	2	130	2
236	2.1	54	1	102	1
		52	2	100	2
246	6.1	86	1	250	1
		184	2	250	2
246	5.3	36	1	84	1
		32	2	86	2
228	14.3	84	1	204	1
		168	2	200	2
238	2.5	80	1	104	1
		80	2	106	2
Avg 240.2		57.0	1	150	1
		83.6	2	154.4	2

10^{-5} and 10^{-9} . The changes occurring in each case after the application of the drug were definite in character and were of sufficient magnitude to preclude any conclusions pertaining to the action of acetylcholine on the cardiac activity of this animal.

Physostigmine. If the acetylcholine is the chemical mediator between nerve terminations and the cardiac muscle in *Daphnia magna*, it should be possible to demonstrate this by the appropriate administration of physostigmine, for the protective action of physostigmine on acetylcholine might lead to increased heart activity. When individuals were eserinizated for a period which varied from 5 to 15 minutes and after this, cardiac inhibition was produced in the usual way mechanically, it was observed that immediately after escape from inhibition, the cardiac rhythm became regular and there was a quick reestablishment of the normal rate of heart beat, without treating the animals with acetylcholine. The action of physostigmine 10^{-4} is shown in Table II. In addition to this a range of other concentrations were employed which varied in strength from 10^{-3} to 10^{-7} and the observations recorded were similar to those shown in Table II. This rapid recovery of the cardiac activity of eserinizated individuals is in marked contrast with the situation found in animals which were not (previous to the arrest of the heart) treated with physostigmine (Table I, column 3).

In the course of the eserinization of the animals there was also observed an acceleration of the heart beat but the action of physostigmine was not immediate. The rate of heart beat under the influence of physostigmine 10^{-4} , before cardiac inhibition presented in Table II does not represent the maximum cardiac activity under the influence of this drug.

Discussion. Repeated endeavors by staining methods to demonstrate clearly the nerve supply to the heart of *Daphnia magna* were beset by many difficulties. We are in possession, however, of cer-

TABLE II.
Action of Physostigmine on Recovery of Heart of *Daphnia magna* (First Brood Mothers) from Cardiac Inhibition.
Rate of heart beat expressed in beats per minute.

Normal heart rate	Treatment with Physostigmine 10^{-4} , min.	Rate of beat before heart arrest	Period of heart arrest, min.	Heart rate, 1 min. after recovery
260	10	300	6.7	270
240	10	264	5.7	246
240	15	254	1.7	256
236	5	242	2.2	152
246	10	252	1.7	246
Avg 244.4		262		234

tain facts pertaining to the nervous system of a number of larger Crustacea. Carlson⁸ has demonstrated a pair of nerves arising from the thoracic ganglion of *Palinurus*; one of these when stimulated has been found to inhibit the heart and the other to be acceleratory in function. The functional activity of extrinsic nerves to the heart has been abundantly confirmed also for a number of other Crustacea by this same worker and the nervous elements within the heart have been described and illustrated by Alexandrowicz.⁹ Although there is nothing in Crustacea, insofar as we know, which corresponds morphologically to the autonomic nervous system in vertebrates, it is evident from the observations presented here that the intestine and the heart of *Daphnia magna* are subject to acceleratory and inhibitory nervous influences. In the mammals the parasympathetic transmitter is believed to be identical with acetylcholine and causes cardiac inhibition. The actions of this drug and of physostigmine on the heart of *Daphnia magna* are the direct opposite of their effects in mammals but are in accord with those reported for a number of invertebrates.¹⁰ Artemov and Mitropolitanskaja¹¹ have demonstrated the presence in whole *Daphnia* of an acetylcholine-like substance. As yet, however, no one has undertaken to demonstrate the presence or absence of choline esterase in this group of animals. The questions of how acetylcholine, if present in *Daphnia*, is bound in the tissues and how it is protected, and the question of the mechanisms involved in the specific effects of acetylcholine and physostigmine in invertebrates as those contrasted in vertebrates must wait for further investigations before they are answered.

Summary. The heart of *Daphnia magna* can be arrested by applying a mechanical stimulus to a region in the digestive tube where the stomach enters the intestine. This induced inhibition and likewise the recovery after inhibition, has enabled us to make a study of the action of acetylcholine and physostigmine on the possible control and coördination of the heart beat. If the animal is treated with acetylcholine when the heart beat, after escape from inhibition, is still feeble, slow and irregular, there is observed an immediate acceleration in the heart beat and the cardiac activity becomes more powerful and rhythmic in character. Animals treated with physostigmine previous to the arrest of the heart, show complete restoration of the regular beat soon after the escape from inhibition.

⁸ Carlson, A. J., *Am. J. Physiol.*, 1905, **15**, 127.

⁹ Alexandrowicz, J. S., *Quart. J. Micro. Sci.*, 1932, **75**, 18.

¹⁰ Walsh, J. H., *Physiol. Zool.*, 1939, **12**, 231.

¹¹ Artemov, N. M., and Mitropolitanskaja, R. L., *Bull. de Biol. et de Méd. Exp. U.R.S.S.*, 1938, **5**, 378.