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PHYSIOLOGICAL INVESTIGATIONS INTO THE HEART FUNCTION OF *DAPHNIA*

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Previous work of Krijgsman & Krijgsman-Berger (1950, 1951) indicates that the automatism of the heart of insects is a neurogenic mechanism which is initiated in the cells of the lateral heart nerves. This automatic nerve centre, which shows adrenergic properties, is influenced by extra-cardiac excitatory nerves, which stimulate the centre by release of acetylcholine. Hence, though itself entirely different from the myogenic 'pacemaker' of the vertebrate heart, this neurogenic pacemaker has some properties in common with the autonomic nerve system of vertebrate animals. From the experiments of numerous authors on several species of Crustacea, which have been reviewed by Welsh & Schallek (1946) and by Krijgsman & Krijgsman-Berger (1951), it can be taken that the initiation of the heart beat in most arthropods is neurogenic.

There are, however, exceptions. Baylor (1942) found that the heart of *Daphnia* is not, as in other arthropods, stimulated by acetylcholine and eserine, but inhibited. According to him atropine also causes an inhibition. Prosser (1942) reports that acetylcholine and adrenaline have no effect on the heart of *Artemia*, and that acetylcholine is also ineffective on the heart of *Eubranchipus*. Needham (1950), who studied the effect of ether on *Daphnia*, shares the opinion that this heart and also the hearts of *Simocephalus*, of *Artemia* and of some larval insects are all myogenic. Histological support of this point of view was provided by Ingle (cited by Prosser, 1942), who found no ganglion cells in the heart of *Daphnia*.

Since the response to drugs may be characteristic for the nature of the pacemaker and since, so far, only a few drugs have been used to investigate the mechanism of the heart in *Daphnia*, it seemed useful to repeat the experiments of Baylor and to extend our knowledge on this subject by studying the influence of other drugs so as to be able to compare the results with the action of these substances on the hearts of other arthropods and of vertebrates.

METHODS

We used *D. magna* and *D. pulex*, which were stocked in an aquarium. No differences were observed in the reactions of the hearts of these two species. Baylor (1942) studied the action of drugs at 10° C., thus lowering the frequency to a measurable rate, and keeping the animals entangled in cotton-wool on a slide. He sucked off the water and replaced it by the required drug solution. We put some *Daphnia* into a narrow glass tube which was mounted on the stage of a microscope. The animals were kept motionless between fibres of cotton-wool, so that the heart could be easily studied under low power. One side of the tube was connected to a two-way stopcock by means of which either water or the required solution could be led through the tube. These solutions, supplied from reservoirs, ran through a thermostat kept at 10° C., before they entered the experimental tube. The rate of flow was kept constant by means of a screw clamp. The distance between thermostat and experimental tube was short, so that the temperature in the experimental tube was constant enough to allow for a suitable and fairly constant heart frequency. This was checked by control experiments with the animals in water. The results showed that there were variations between individual animals, probably related to body size and sex. The individual rhythm, however, was fairly constant and was maintained for hours under our experimental conditions. In control experiments with nine animals, extended over 150 min., the frequency per minute in an individual animal varied, at most, 7·6%. Taking into account that, in all experiments with drugs, every animal was observed for a control period of 40 min. for constancy of heart rhythm before the drug was applied, and since, moreover, in most cases the results were given as the average of several animals, it appears quite reasonable to consider changes of frequency greater than 10% as significant. In those experiments in which the effect on the heart showed individual differences in the latent period, graphs of single experiments are given as an example, since the average flattened the graphs and thus did not reflect the correct type of the response. Frequency was measured by visual counting with the aid of a stopwatch for 1 min., at intervals of 5–10 min. Frequencies higher than 250 per min., which occurred in a few cases, could not be followed accurately, and these figures therefore only have an approximate value.

Besides frequency, the type of contraction was observed. Irregularity of rhythm could be clearly discriminated; the size of contraction, however, was more difficult to judge. We could discriminate between normal and increased force of beat (termed 'strong'), and contractions in dilated position (termed 'weak').

We tested the effect of acetylcholine (ACh), tetraethylpyrophosphate (TEPP, 95% pure), pilocarpine, atropine, adrenaline, digitalin and rotenone. Each drug was tested on twenty to twenty-six animals. The aqueous solutions of all the drugs (except TEPP) were made from solid crystalline substances.

In carrying out experiments with whole animals which are immersed in a drug solution, it must be realized that the action of the drug is conditioned by the permeability of the surface tissues of the animal. Consequently, the length of the latent period (interval between application and effect) is useless for the comparison of the effect of different drugs, and for the same reason the threshold concentrations are of no value for comparison with other drugs and other species.

RESULTS

Acetylcholine

Acetylcholine caused slowing of the heart of *Daphnia*; acceleration as observed by Obreshkove (1942), who tested ACh on hearts recovering from inhibition produced by mechanical stimulation, was never obtained. A concentration of 1×10^{-8} to 1×10^{-7} was slightly above threshold and produced a progressively increasing slowing of the heart, so that after about 1 hr. immersion the frequency had decreased by about 25%. With higher concentrations the effect became more pronounced; after 90 min. immersion in 5×10^{-7} the frequency

had decreased by about 65%. There were also some irregularities in heart beat after about 15 min. immersion in these higher concentrations and in three experiments out of nine, diastolic arrest occurred later on. Fig. 1 shows the average effect on five hearts with ACh, 1×10^{-7} and 5×10^{-7}.

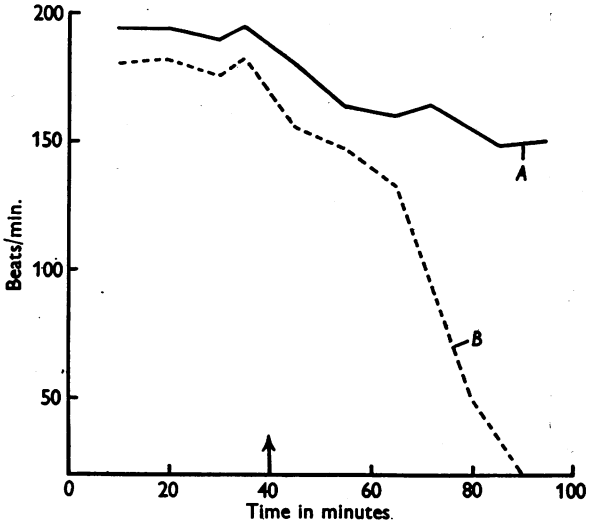

Fig. 1. Action of acetylcholine on *Daphnia* heart. Frequency of heart beat plotted against time. Arrow: moment of application. *A*: 1×10^{-7}, average of five experiments; *B*: 5×10^{-7}, average of five experiments.

Our results, which confirm those of Baylor (1942), show that the heart of *Daphnia* is very sensitive to ACh, but its response is different from those of most other arthropods, which are stimulated by ACh.

Tetraethylpyrophosphate

The effect was similar to that of ACh, as could be expected by the anti-cholinesterase properties of TEPP. The threshold concentration was about $3{\cdot}7 \times 10^{-7}$; double that concentration decreased the frequency by about 35% in the course of 50 min. immersion. The slowing was not generally associated with any visible change in the strength of the heart beat; in two out of ten animals the beat became stronger. A concentration of $1{\cdot}5 \times 10^{-4}$ or 3×10^{-4} of TEPP caused pronounced slowing within a few minutes, and a decrease in frequency of about 80% within 30–40 min. The average effect of these concentrations of TEPP is shown in Fig. 2.

Our results are in keeping with those Baylor (1942) obtained with eserine, but not with those obtained by Obreshkove (1942), who found a stimulating effect with eserine.

Pilocarpine

Pilocarpine also slowed the heart. 1×10^{-5} was ineffective; 5×10^{-5} was about threshold, causing a slowing in four out of seven hearts; after prolonged immersion irregularities of heart beat were observed in one case. The effect of these

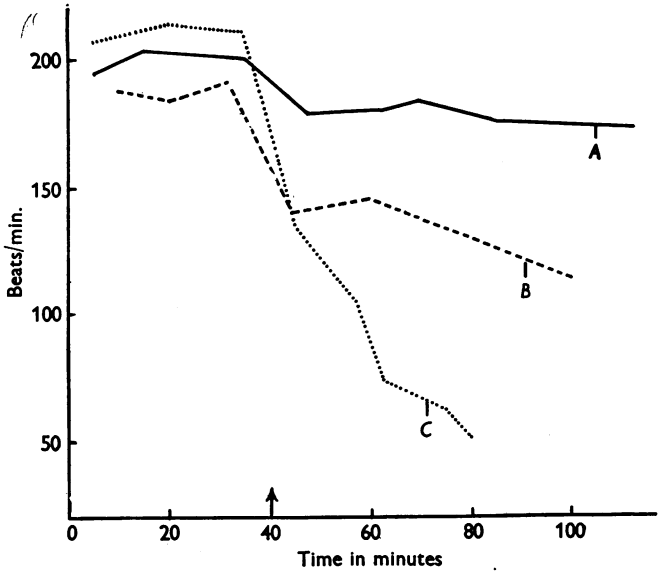

Fig. 2. Action of TEPP. Frequency of heart beat plotted against time. Arrow: moment of application. *A*: $3{\cdot}7 \times 10^{-5}$, average of seven experiments; *B*: $7{\cdot}5 \times 10^{-5}$, average of four experiments; *C*: 3×10^{-4}, average of four experiments.

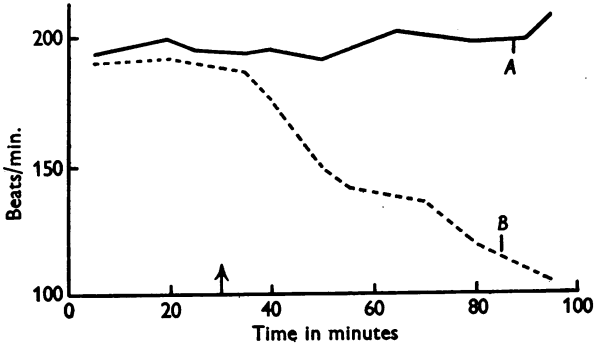

Fig. 3. Action of pilocarpine. Frequency of heart beat plotted against time. Arrow: moment of application. *A*: 1×10^{-5}, average of three experiments; *B*: 5×10^{-5}.

two concentrations of pilocarpine is shown in Fig. 3. Higher concentrations showed no change in the effect. Pilocarpine never stimulated the heart of *Daphnia*, which thus responds differently to those of other arthropods, which are stimulated by it.

Atropine

Atropine accelerated the heart. A concentration of $6{\cdot}25 \times 10^{-5}$ was sometimes effective causing an increase in frequency up to 40%. The effect was always a temporary one; the frequency returned to normal within 50 min. A greater concentration of atropine, say $2{\cdot}5 \times 10^{-4}$, produced a more pronounced and longer-lasting acceleration (Fig. 4). With still higher concentrations, there occurred, after an initial great acceleration, slowing of the heart in two out of seven experiments, with a tendency to weak contractions. Our results differ from those of Baylor (1942), who did not observe any acceleration with atropine, but inhibition only.

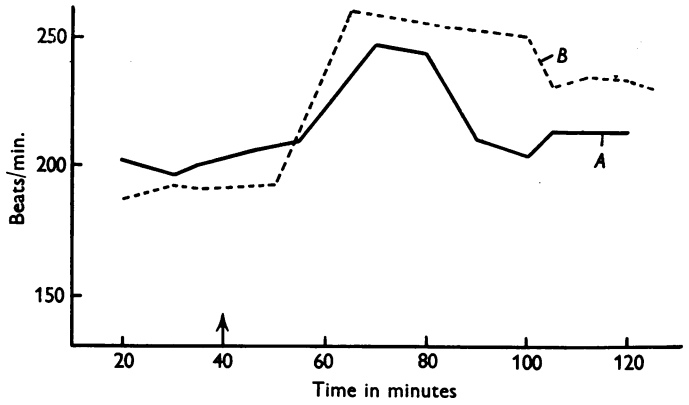

Fig. 4. Action of atropine. Frequency of heart beat plotted against time. Arrow: moment of application. *A*: $6{\cdot}25 \times 10^{-5}$; *B*: $2{\cdot}5 \times 10^{-4}$.

Adrenaline

Adrenaline was found to slow the heart when tested in low, and to accelerate it when tested in high, concentration. A concentration of 2×10^{-7} was about threshold; five animals were not affected by it; in three others the frequency was decreased by about 30% in the course of an hour's immersion. A concentration of 2×10^{-6} caused slowing in all hearts; the frequency decreased by about 45% in the course of an hour's immersion. In one case the beat became irregular and the heart stopped. A concentration of 2×10^{-5} was either ineffective or it slightly increased the frequency, and in one heart it caused some slowing. A concentration of $1{\cdot}25 \times 10^{-4}$ caused in six hearts a pronounced but temporary acceleration sometimes accompanied by stronger beats. One heart was slightly slowed by this concentration of adrenaline. The average effect of these concentrations is shown in Fig. 5. Our results differ from those obtained by Baylor (1942), who found only an acceleration of the *Daphnia* heart with concentrations of adrenaline as low as 1×10^{-7}. The usual response of the heart of other arthropods to adrenaline is acceleration.

Digitalin

Digitalin causes in *Periplaneta* (Krijgsman & Krijgsman-Berger, 1951) temporary acceleration with increased amplitude of contraction, when given in low concentration, and subsequently slowing and even arrest, when given

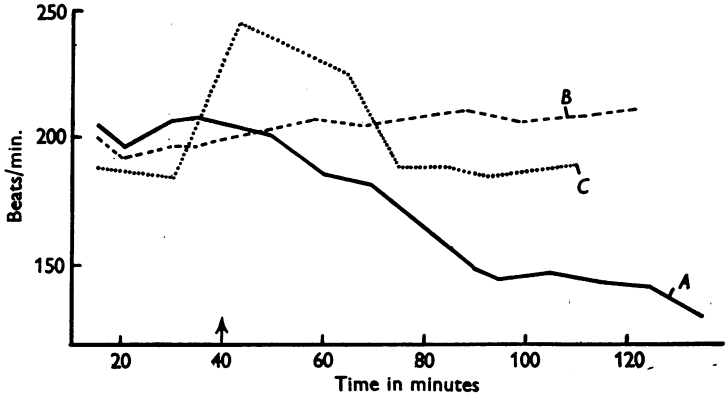

Fig. 5. Action of adrenaline. Frequency of heart beat plotted against time. Arrow: moment of application. A: 2×10^{-6}, average of five experiments; B: 2×10^{-5}, average of five experiments; C: $1{\cdot}25 \times 10^{-4}$, average of six experiments.

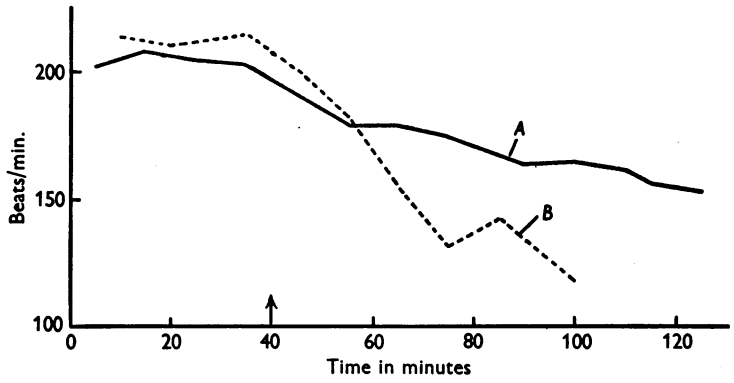

Fig. 6. Action of digitalin. Frequency of heart beat plotted against time. Arrow: moment of application. A: 5×10^{-6}, average of four experiments; B: 5×10^{-5}, average of five experiments.

in high concentration. In *Daphnia* it caused only slowing. Concentrations below 5×10^{-6} were ineffective; 5×10^{-6} caused a progressive slowing, so that the frequency decreased by about 30% in the course of 80 min. immersion. In two hearts there was a tendency to weak contractions towards the end of the experiment. A concentration of 5×10^{-5} or more caused a more pronounced slowing which developed quickly; irregular and weak contractions occurred at the end of the experiment. Fig. 6 shows the average effect of digitalin, 5×10^{-6} and 5×10^{-5}.

Rotenone

Rotenone, which is relatively inactive on the vertebrate heart, causes, in low concentration, inhibition of the heart of *Periplaneta*; the beat becomes slower and weaker (Krijgsman, Dresden & Berger, 1950). It has the same action on the heart of *Daphnia*. A concentration of $2{\cdot}5 \times 10^{-8}$ was about threshold, causing

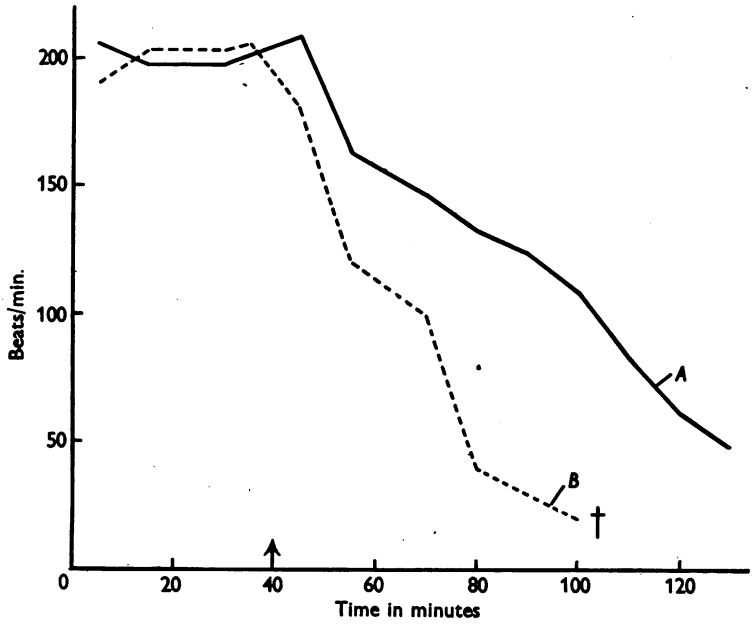

Fig. 7. Action of rotenone. Frequency of heart beat plotted against time. Arrow: moment of application. *A*: 1×10^{-7}, average of seven experiments; *B*: $1{\cdot}5 \times 10^{-6}$, average of five experiments. †: arrest.

a gradually progressive slowing, so that the frequency decreased by about 30% during 45 min. immersion. With stronger concentrations the effect became more pronounced. These concentrations caused irregularities and arrest in diastole after 20–30 min. The average slowing by rotenone, 1×10^{-7} and $1{\cdot}5 \times 10^{-6}$, is shown in Fig. 7.

DISCUSSION

Our results on the response on the heart of *Daphnia* to ACh and the anti-cholinesterase TEPP confirm those of Baylor (1942). They suggest that this heart, unlike that of most other arthropods, has no neurogenic pacemaker. The slowing of the *Daphnia* heart by low concentrations of these drugs is a response characteristic of the myogenic heart of vertebrates and molluscs. We may assume that the myogenic pacemaker of the *Daphnia* heart is normally under the influence of ACh, which is probably released from the endings of extrinsic cholinergic nerves impinging on the pacemaker. The inhibitory effect

of TEPP and of eserine is best explained by the cholinesterase-inhibiting property of these substances, and thus by accumulation of released ACh when its normal enzymatic hydrolysis is prevented.

Pilocarpine, which is known to act in vertebrates like the parasympathetic transmitter, also exerts an inhibitory action on the heart of *Daphnia*. Our results with this drug further support the assumption of a myogenic pacemaker in the *Daphnia* heart.

Atropine has an accelerating action on the *Daphnia* heart, thus supporting the opinion that the heart of this animal is normally under some inhibitory influence of ACh.

Whereas, concerning ACh, anticholinesterases, pilocarpine and atropine, the heart of *Daphnia* behaves like a vertebrate heart, it is difficult to find a satisfactory explanation for the action of adrenaline. The neurogenic heart of most arthropods is accelerated by adrenaline, an effect obtained on the heart of *Daphnia* with high concentrations only; low concentrations caused slowing. This is certainly not the action of adrenaline on the vertebrate heart. Evidence has been obtained, that in vertebrates adrenaline does not always act as an antagonist of acetylcholine (literature reviewed by Burn (1945, 1950)), but sometimes supports the action of the latter. Although such interactions might also play a role in the case of *Daphnia*, it is, on the basis of the data available, unlikely that the accelerating action on the heart of *Daphnia* is of physiological importance, since it occurs only with high concentrations, and it is difficult to imagine a physiological role for the inhibitory effect, once we assume a cholinergic inhibitory extra-cardiac innervation. It may, therefore, well be that adrenaline has no physiological function whatsoever in the heart of *Daphnia*.

The slowing of the *Daphnia* heart caused by digitalin is in contrast to the acceleration produced in the neurogenic heart of *Periplaneta* by low concentration, and this difference may be characteristic for the action of digitalin on the two types of the heart of arthropods. The slowing produced by digitalin may be an effect on the muscle or it may be the result of stimulation of the cholinergic nerves to the pacemaker. We may remember that the initial slowing produced by digitalin in vertebrates is dependent on the integrity of the vagus nerve.

Rotenone acts similarly on the neurogenic heart of *Periplaneta* and on the myogenic heart of *Daphnia*. On both hearts low concentrations of rotenone cause slowing. At the time when its effect on *Periplaneta* was first observed (Krijgsman *et al.* 1950), it was thought to produce inhibition by paralysing the accelerating cholinergic nerves to the neurogenic pacemaker. The present finding, however, of a similar inhibitory action on the heart of *Daphnia*, makes such an interpretation unlikely. The effect of rotenone on both types of heart is therefore probably a direct effect on the heart muscle.

SUMMARY

1. The actions of drugs were studied on the heart of *Daphnia*. For this purpose the animals were put into a narrow glass tube. Water containing the required concentration of the drug under examination was led through the glass tube which was mounted to the stage of a microscope and the heart action observed under low power.

2. Acetylcholine, tetraethylpyrophosphate and pilocarpine slow the heart.

3. Atropine accelerates the heart.

4. Adrenaline in low concentration slows the heart, in high concentration accelerates it.

5. Digitalin and rotenone slow the heart.

6. The results stated in (2) and (3) are in full accordance with the assumption of a myogenic pacemaker in the heart of *Daphnia*, inhibited by extra-cardiac cholinergic nerves.

7. The actions of adrenaline and digitalin also are different from those on the neurogenic heart of most arthropods.

8. Rotenone has the same action on the myogenic as on the neurogenic heart of the arthropods; its action is therefore probably directly on the heart muscle.

REFERENCES

Baylor, E. R. (1942). *Biol. Bull. Wood's Hole*, **83**, 165.
Burn, J. H. (1945). *Physiol. Rev.* **25**, 377.
Burn, J. H. (1950). *Physiol. Rev.* **30**, 177.
Krijgsman, B. J., Dresden, D. & Berger, Nel E. (1950). *Bull. ent. Res.* **41**, 141.
Krijgsman, B. J. & Krijgsman-Berger, Nel E. (1950). *Nature, Lond.*, **165**, 936.
Krijgsman, B. J. & Krijgsman-Berger, Nel E. (1951). *Bull. ent. Res.* **42**, 143.
Needham, A. E. (1950). *Nature, Lond.*, **166**, 9.
Obreshkove, V. (1942). *Proc. Soc. exp. Biol., N.Y.*, **49**, 427.
Prosser, C. L. (1942). *Biol. Bull. Wood's Hole*, **83**, 145.
Welsh, J. H. & Schallek, W. (1946). *Physiol. Rev.* **26**, 447.