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Abstract

On the median nerve trunk-heart muscle preparation of Limulus the authors studied the effects of atropine and acetylcholine upon the pace maker ganglion cells. The results are summarized as follows: (1) Atropine exerts an excitatory action on the pace maker ganglion cells in a concentration of 1-2 per cent. resulting in an increase of the heart rate. No effect is recognized on the heart beats, where the drug is applied to the heart muscle. (2) Acetylcholine exerts an excitatory action in a lower concentration (0.001-0.10 %) and produces a transitory excitation followed by an inhibition in a higher concentration (1-5 %). No effect is perceptible on the heart beats, when the drug is applied to the heart muscle. (3) Where atropine has been previously applied to the median nerve trunk, acetylcholine applied to the same spot produces always an inhibition of the heart beats. Conversely, when the ganglion cells activated previously by acetylcholine, a subsequent administration of atropine suppresses the activity of the ganglion cells, resulting in an inhibition of the heart beats.

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THE ACTION OF ATROPINE AND ACETYLCHOLINE ON THE PACE MAKER GANGLION CELLS OF LIMULUS HEART

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HUKUHARA *et al*[8,9]. showed that in rabbits the motility of the excised strip of the small intestine which is immersed in the warmed TYRODE's solution, is, in most of cases, capable of being excited by atropine or acetylcholine when administered singly, whereas it is always inhibited by successive administration of different drugs, regardless of the order of administration. Recently a similar phenomenon was also observed on the pace maker ganglion cells of *Limulus* heart. The results are presented in the following.

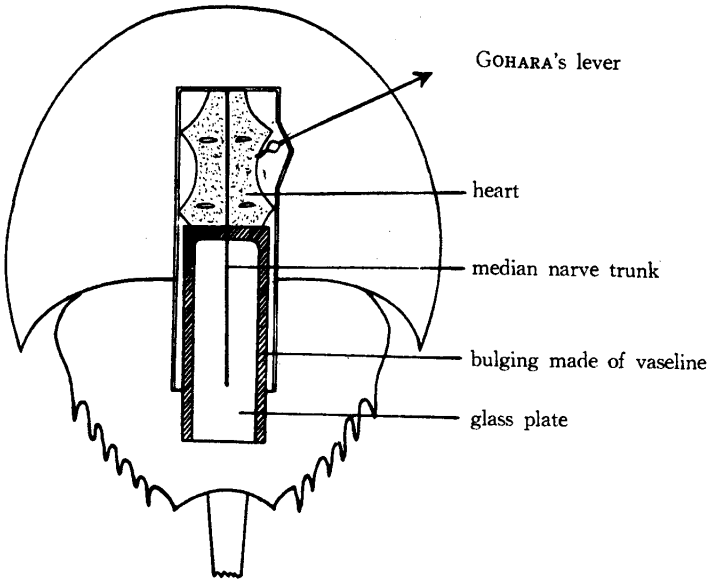
METHODS

The experiments have been carried out in October, 1956 on 35 *Limulus longispina Hoeven* (*Tachypleus tridentatus Leach*). The animal is fixed in prone position. The heart is exposed by removing the carapace and dorsal muscles dorsal to the heart. The median ganglionic trunk from its caudal end to the level of the third segment is carefully isolated from the heart muscles, nervous connections with the lateral nerves being severed. And a glass plate (2×10 cm), the anterior and lateral borders of which are fringed with the bulging made of vaseline, is laid under the isolated trunk (Fig. 1). By these procedures the action of the drug in solution can be confined either to the heart ganglion or to the anterior segments. The preparation described above can be regarded as a pace maker ganglion cells-heart muscle preparation, since there exist practically no rhythmic nerve cells in the first two segments[1,2,3]. The lateral border of the second segment is connected by means of a serrefine and a thread to GOHARA's lever to record the heart beats on the surface of the drum. The drugs used are acetylcholine chloride (*Roche*) and atropine sulfate (Japanese pharmacopoeia), being dissolved in sea water. The former is prepared in solution immediately before the use.

RESULTS

When the cotton wool soaked with sea water to which atropine is added in the concentration of 1—5 per cent is applied to the median nerve trunk, there occurs a remarkable increase of cardiac rate, while the height of contractions is not changed at all or increases only slightly and decreases occasionally (Fig. 3). When, on the contrary, atropine of the same concentration as above is applied

Fig. 1. The pace maker ganglion cells-heart muscle preparation of *Limulus*.

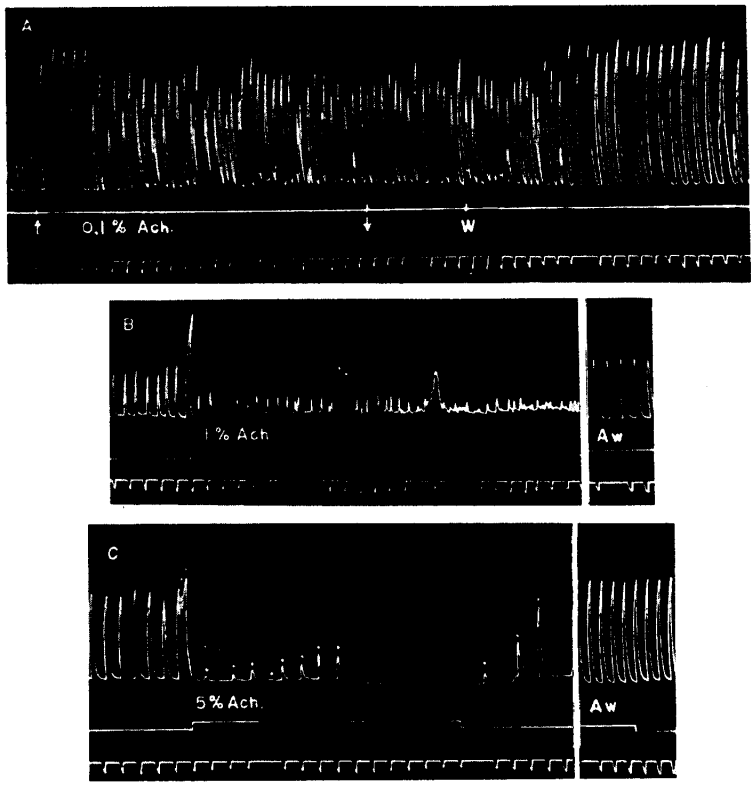


to the cardiac muscle, no change is to be seen on the cardiac beats.

When the cotton wool soaked with sea water supplemented with acetylcholine in the concentration of 0.001—0.10 per cent is applied to the isolated part of the median nerve trunk, the rate of heart beats increases remarkably and becomes somewhat irregular, being accompanied by a slight reduction and irregularity of amplitude of contractions as shown in Fig. 2 A. By applying a stronger acetylcholine solution (1 %) the irregularity of rate and amplitude of contractions becomes far more remarkable, one or two weak contractions being interpolated between two relatively stronger contractions (Fig. 2 B). In this case the heart apparently shows the aspect of fibrillation. A further stronger acetylcholine (5 %) produces a transient tetanus followed by an inhibition of the beats, i. e., a remarkable decrease of rate and amplitude of contractions (Fig. 3 C). By rinsing the nerve trunk repeatedly with sea water the normal beats are recovered again. When, on the other hand, acetylcholine of various concentrations is applied to the heart muscle, no effect is brought about on the movements of the heart. From the results described above it may be concluded that acetylcholine elicits the excitation of the pace maker ganglion cells in a lower concentration, while it elicits an inhibition in a higher concentration.

When atropine and acetylcholine are applied to the pace maker ganglion cells in a concentration capable of producing an accelerating effect upon the ganglion cells, curious phenomenon occurs: The accelerating effect is not only

Fig. 2. The effects of acetylcholine on *Limulus* pace maker ganglion cells.

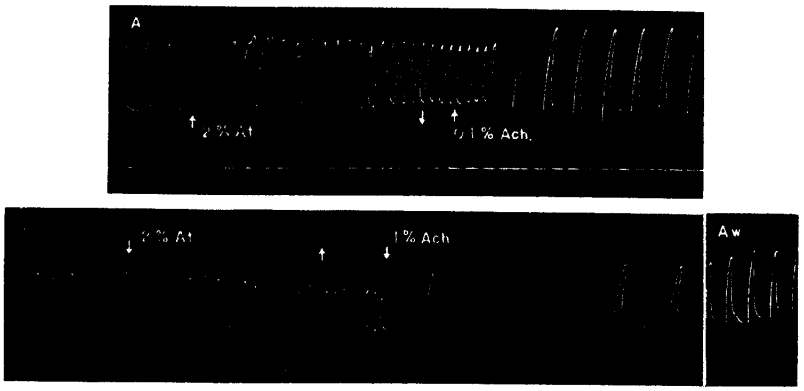


Acetylcholine acts as a stimulant on the ganglion cells in a lower concentration (A), while it acts as an inhibitor in a higher concentration (B and C). W : washing. Aw : after washing. Time in 5 sec. intervals.

suppressed, but also may be reversed to the inhibitory one. The example is shown in Fig. 3—A and B: When the heart beats are accelerated by application of atropine on the ganglion cells, the subsequent application of acetylcholine always reduces the rate of beats remarkably and occasionally stops the beats completely. The higher the concentration of atropine, the more remarkable the inhibition of the beats becomes. It is to be noted that the beats apparently recover to the normal level when atropine has been rinsed off.

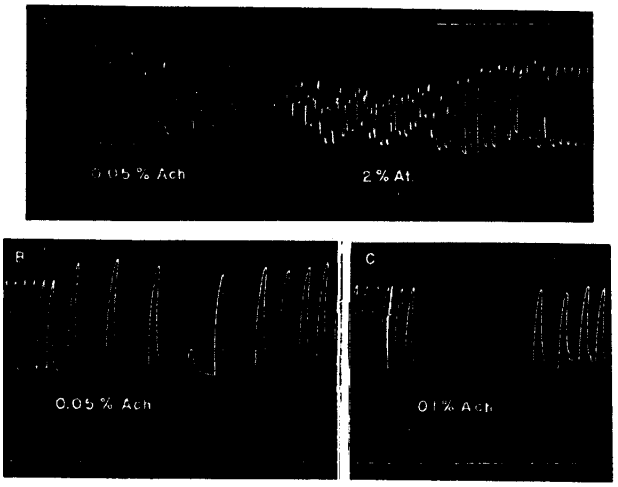
When at first acetylcholine and then atropine are applied to the median nerve trunk, the reversal phenomenon similar to the case described above can also be seen, as shown in Fig. 4 A. In this case, however, the inhibition is less remarkable than in the former case. When once atropine is applied, during the period where the beats have been apparently restored to normal, the subsequent application of acetylcholine does not exert any excitatory action, but always an

Fig. 3. The effects of acetylcholine on *Limulus* pace maker ganglion cells activated previously by atropine.



When atropine is applied to the median nerve trunk, the rate of heart beats is accelerated and the subsequent application of acetylcholine reduces the rate of beats (A) and occasionally stops the beats completely (B). Aw : after washing. Time in 5 sec. intervals.

Fig. 4. The interaction of atropine and acetylcholine on *Limulus* pace maker ganglion cells.



The application of acetylcholine to the median nerve trunk increases remarkably the rate of heart beats which is reduced by the subsequent application of larger dose of atropine (A). Subsequent application of acetylcholine produces always an inhibitory action (B and C).

inhibitory one (Fig. 4—B and C).

DISCUSSION

It has been established by GASSER[6,11] that acetylcholine exerts in general an

excitatory action on the ganglion cells residing in the median nerve trunk. Atropine acts also as a stimulant on the cardiac ganglion cells of *Limulus*[4,6]. And GARREY[6] shows that atropine does not block the action of acetylcholine but summates with that of the latter. In the foregoing experiments we have found a curious phenomenon in relation to the interaction of atropine and acetylcholine: Both drugs, atropine and acetylcholine, act as stimulants on the cardiac ganglion cells, provided each is administered alone in a moderate concentration, whereas either one of the drugs produces an inhibition of the ganglion cells, where any other drug has been previously administered. Such a phenomenon has not been mentioned by any previous authors. The mechanism that gives rise to such a phenomenon is unknown. It is, however, noted that a similar phenomenon can also be observed in the small intestine of dogs and rabbits: HUKUHARA *et al.*[8,9] observed that the small intestine, both *in situ* and *in vitro*, is capable of being excited by atropine and such an excitatory state is suppressed and changed in the inhibitory one by acetylcholine, and that, *vice versa*, the excitation produced by acetylcholine is converted to the inhibition by atropine. It is clearly demonstrated by MAGNUS[10], GASSER[7] and especially by *van* ESVELD[5] that atropine exerts always an excitatory action on the motility of the strips of the longitudinal muscle containing AUERBACH's plexus, whereas that of the ganglion free circular muscle strips is always inhibited. From their results it may be concluded that atropine stimulates the ganglion cells in the plexus to give rise to the excitation of the intestinal motility. It may be thus presumed that this curious phenommenon observed in the small intestine is identical with that observed in the heart ganglion of *Limulus* in its basic mechanism.

CONCLUSIONS

On the median nerve trunk-heart muscle preparation of *Limulus* the authors studied the effects of atropine and acetylcholine upon the pace maker ganglion cells. The results are summarized as follows:

(1) Atropine exerts an excitatory action on the pace maker ganglion cells in a concentration of 1—2 per cent, resulting in an increase of the heart rate. No effect is recognized on the heart beats, where the drug is applied to the heart muscle.

(2) Acetylcholine exerts an excitatory action in a lower concentration (0.001—0.10 %) and produces a transitory excitation followed by an inhibition in a higher concentration (1—5%). No effect is perceptible on the heart beats, when the drug is applied to the heart muscle.

(3) Where atropine has been previously applied to the median nerve trunk, acetylcholine applied to the same spot produces always an inhibition of the heart

beats. Conversely, when the ganglion cells activated previously by acetylcholine, a subsequent administration of atropine suppresses the activity of the ganglion cells, resulting in an inhibition of the heart beats.

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