PHARMACOLOGICAL INVESTIGATION OF THE NATURE OF THE INNERVATION OF THE MUSCLES OF HELIX ASPERSA

BY JAS. D. P. GRAHAM AND THE LATE R. ST A. HEATHCOTE
Department of Pharmacology, Welsh National School of Medicine, Cardiff

(Received 30 July 1951)

(With Two Text-figures)

Bacq (1947) has reviewed the known actions of autonomic effector substances on invertebrates. Concerning the muscles of snails relatively little is known. Boyer (1926) showed that adrenaline had no effect on the contraction of the ventricular muscle of Helix pomatia in a concentration of less than 1 in 20,000, but that higher concentrations slowed the heart and produced irregularities of rhythm. Very high concentrations (1 in 250) arrested the heart beat. According to Jullien (1936) the ventricle of the same snail is sensitive to acetylcholine in a concentration of 1 part per million. There seems to be little other information available, other than the general statements of Bacq (1947) that in many Molluscs physostigmine (eserine) fails to potentiate the effects of acetylcholine, that in Cephalopods curare, but not atropine, abolishes the effect of stimulating the vesical nerve, and that in Molluscs as a whole the muscles of locomotion do not appear to be cholinergic in type.

In the common English snail, Helix aspersa, a portion of the alimentary canal (crop and rectum), or of the heart (ventricle), or the columellar muscle (which retracts the main part of the snail within its shell) was isolated, and the effects of drugs upon it examined, with the aim of determining the nature of the neuro-hormonal mechanism involved in contraction.

METHOD

Large snails were collected and kept in a cool vessel upon fresh leaves. When a snail was required the shell was opened, the foot pinned out, and the columellar muscle, the cardiac ventricle, the rectum or the crop excised. The latter organ was freed of salivary glands, and care was taken to remove it caudal to the ganglia so as to obtain a nerve-free preparation. The organ was suspended between fine nickel hooks in a bath at 18°C., oxygenated, and containing a fluid of the following composition (after Boyer, 1926): NaCl 6·5, KCl 0·14, CaCl₂ 0·12, NaHCO₃ 0·01, NaH₂PO₄ 0·01, in g. per litre. One end of the tissue was attached to a gimbal lever recording on a kymograph. Drugs were added in solution in the nutrient fluid. Histological preparations were made of the organs used.
Neuro-hormones of Helix aspersa muscle

RESULTS

The fibres of the columellar muscle and in the heart and the alimentary canal resembled vertebrate smooth muscle in general structure. The results obtained after addition of adrenaline HCl, ephedrine HCl, acetylcholine Br, physostigmine salicylate, atropine SO₄, d-tubocurarine, nicotine tartrate, barium Cl₂, magnesium Cl₂, and veratrine are shown in Table 1.

Table 1. Actions of drugs on muscle from Helix aspersa
(+ means contraction, — means relaxation, o means no observed effect with a concentration of $10^{-4}$, . . . means drug not tested.)

<table>
<thead>
<tr>
<th>Drug, minimal effective conc. and action</th>
<th>Preparation</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Columellar muscle</td>
</tr>
<tr>
<td>Adrenaline $10^{-4}$—$10^{-7}$ +</td>
<td>$10^{-6}$ +</td>
</tr>
<tr>
<td>Ephedrine $10^{-3}$ +</td>
<td>$10^{-5}$ +</td>
</tr>
<tr>
<td>Acetylcholine $10^{-4}$ +</td>
<td>$2 	imes 10^{-4}$ +</td>
</tr>
<tr>
<td>A.Ch. after eserine $10^{-7}$ +</td>
<td>$10^{-6}$ +</td>
</tr>
<tr>
<td>A.Ch. after atropine $10^{-4}$ +</td>
<td>$10^{-5}$ +</td>
</tr>
<tr>
<td>Nicotine $10^{-4}$ +</td>
<td>$10^{-5}$ +</td>
</tr>
<tr>
<td>Barium $10^{-4}$ +</td>
<td>$10^{-5}$ +</td>
</tr>
<tr>
<td>Magnesium $10^{-4}$ +</td>
<td>$10^{-5}$ +</td>
</tr>
<tr>
<td>Veratrine $10^{-3}$ +</td>
<td>$10^{-4}$ +</td>
</tr>
</tbody>
</table>

Potentiation of the effects of acetylcholine following pre-treatment with eserine was clearly visible in the columellar muscle preparation, and to a lesser extent with the crop and rectum and is illustrated in Fig. 1. Abolition of the response to acetylcholine after pre-treatment with atropine occurred if the latter compound was left in contact with the muscle for a prolonged period (10–20 min.) in a concentration of $10^{-4}$ but not if the acetylcholine was added only 2 or 3 min. after the atropine. At $10^{-4}$ atropine was quickly effective. Curare ($10^{-4}$ for 20 min.) failed to affect the action of acetylcholine.

Acetylcholine had no effect on the ventricle, but it contracted the crop and rectum (weak action), and this effect also was potentiated by eserine, and reduced or abolished by atropine. Adrenaline had a stimulant action on the columellar muscle, stimulated the rectum and crop (weak action), and slowed the rate of contraction of the ventricle (weak action). These effects were not potentiated by cocaine. Ephedrine also contracted the columellar muscle and affected the ventricle, producing a slower beat and a larger individual contraction. Nicotine exerted no effect on the preparations, which were free of ganglia. The two ions, Ba and Mg, which have a direct action on muscle, had the same effects as in vertebrate preparations, and veratrine caused its customary spasmodic contraction. This, however, was reversible after washing (see Fig. 2). The stimulation of the columellar muscle with cholinergic and sympathomimetic drugs was constant and reproducible, as was that with Ba⁺⁺ and veratrine.
Fig. 1. Isolated columnar muscle of *Helix aspersa*. Acetylcholine $10^{-4}$ causes contraction (1) which is potentiated (3) by the previous addition (2) of eserine $10^{-4}$.

Fig. 2. Isolated columnar muscle of *Helix aspersa*. Adrenaline $10^{-4}$ causes a repetitive contraction (1). Veratrine $10^{-4}$ causes a spasm which is reversible on washing (2). Barium $10^{-4}$ causes a contraction (3).

**DISCUSSION**

The motor response of the columnar muscle to adrenaline and acetylcholine is curious, but has been observed in higher forms of life (intestine of duck, uterus and ileum of guinea-pig under certain conditions, nictitating membrane of cat). The explanation in the snail may lie in the nature of the response to the two drugs, which differs in detail. The reaction to added adrenaline is a vigorous contraction of rapid onset and short duration, sometimes repetitive in nature. Acetylcholine, on
the other hand, usually causes a prolonged spasm. These two types of reaction may be related to differences in function. The potentiation of the actions of acetylcholine by physostigmine suggests that the tissue contains cholinesterase which acts as the normal mechanism of destruction of acetylcholine, and the action of atropine in dilute solution \((10^{-6})\) in abolishing the action of acetylcholine tends to confirm the supposition that this muscle has a cholinergic muscarinic innervation like much of the smooth muscle of vertebrates. Nevertheless, the properties as well as the appearance of the muscle differ markedly from that of vertebrate smooth muscle. The evidence for cholinergic innervation of the alimentary canal is much less strong since acetylcholine only acts on muscles from this site in the 'unphysiological' concentration of \(10^{-4}\) or more, and its effects are much less clear cut than on the columnellar muscle. Nevertheless, physostigmine potentiates these actions, which are abolished by atropine in large doses. No evidence of cholinergic innervation of the heart could be obtained, but by contrast the sympathomimetic compounds adrenaline and ephedrine affected it. They caused a slowing of rhythm, and in the case of ephedrine an increased vigour of contraction. The effects of adrenaline on the preparations from the alimentary canal were too weak and uncertain for any firm conclusion to be drawn, but it is doubtful if any 'autonomic innervation' as we know it in vertebrates is present. The absence of any effect from adding nicotine is not surprising since the preparation is not sensitive to the more potent substances adrenaline and acetylcholine, and is thought to be free of nerve cells. The spasm caused by veratrine was surprisingly easily washed out, in contrast with that seen after veratrine poisoning of vertebrate striped muscle.

**SUMMARY**

The isolated columnellar muscle of *Helix aspersa* is caused to contract by addition to it of ephedrine, adrenaline, veratrine, \(\text{Ba}^{++}\) and acetylcholine.

Adrenaline and acetylcholine are active in concentrations of \(10^{-6}\) or less.

The action of acetylcholine is potentiated by eserine and abolished by atropine, but not by curare, indicating a muscarinic cholinergic innervation of this muscle.

The heart muscle is sensitive to sympathomimetic substances only.

The muscle of the alimentary canal is insensitive to drugs.

**REFERENCES**

