EFFECTS OF EXTRACTS OF ADRENERGIC FIBERS ON THE FROG HEART

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The early observations on the effects of nerve extracts, made by Cleghorn (1899, 1900), were repeated and criticized by Halliburton (1901 a, b). A new interest in this field was awakened by the discovery of chemical mediations of nerve impulses. Numerous investigators began to study the acetylcholine content of different nerves and ganglia (Witanowski, 1925; Plattner, 1933; Chang and Gaddum, 1933; Binet and Minz, 1934; Kwiatkowski, 1935; Barsoum, 1935; Brown and Feldberg, 1936). From the results of experiments carried out in these investigations it appears clear that all motor, parasympathetic and preganglionic sympathetic nerves and also sympathetic ganglia contain acetylcholine in different amounts. Evidence has accumulated that nerve impulses delivered by these nerves discharge acetylcholine at their terminals. The postganglionic sympathetic fibers with few exceptions liberate an adrenaline-like substance at their endings. Dale (1933) proposed that fibers liberating acetylcholine be named cholinergic and that postganglionic sympathetic fibers liberating an adrenaline-like mediator be named adrenergic. Whether the post-ganglionic adrenergic fibers contain acetylcholine or adrenaline had not been determined. It was suggested by Doctor Cannon that I undertake a study of this question.

Method. Cats, rabbits and dogs anesthetized with dial (Ciba, 0.6 to 0.8 cc. per kgm., intraperitoneally) were used. The nerves to be examined were isolated and removed, washed in salt solution, dried with filter paper, then weighed on a torsion balance, afterwards minced and ground up with sea sand (Merck) in a mortar with bicarbonate-free Ringer’s solution (about 1 cc. per 100 mgm. nerve). The extracts were dialyzed, through a cellophane membrane, against an equal amount of bicarbonate-free Ringer’s solution, according to the method of Loewi (1936), for 3 hours in a shaking-machine. The dialysate was tested on isolated frog hearts, according to the method of Straub. When used for testing for positively acting substances, the hearts were rendered hypodynamic by frequent

1 A preliminary publication appeared in Science 88: 434, 1938.
2 Rockefeller Fellow from Hungary.
washing with Ringer’s solution for 3 to 4 hours before the testing procedures were carried out. Such hearts are very sensitive to adrenaline; 0.001γ is easily detectable. If acetylcholine and the adrenaline-like substance are both present in an extract, and a “normal” (i.e., not hypodynamic) heart serves as the test object, the response is the negative effect; there is a decrease of amplitude and rate of the heart, typical of acetylcholine. For quantitative comparison titrations with acetylcholine and with adrenaline were made on the same heart.

The drugs used were: adrenalin (Parke, Davis), acetylcholine (Merck), ergotoxine ethanesulfonate (Burroughs, Wellcome), and physostigmine salicylate (Merck).

RESULTS. A. Extracts of different nerves in Ringer’s solution plus physostigmine. Extracts were made from the vagus, cervical sympathetic, sciatic, phrenic, thoracic sympathetic chain, mesenteric plexus and the

| TABLE 1 |
| Acetylcholine equivalent of extracts in γ per gram nerve |
| Average values calculated from 10 experiments. The maximal deviation from the average was 30 per cent. |

<table>
<thead>
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<th>NERVES</th>
<th>CATS</th>
<th>DOGS</th>
<th>RABBITS</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cervical vagus</td>
<td>10</td>
<td>8</td>
<td>8</td>
</tr>
<tr>
<td>Cervical sympathetic trunk</td>
<td>11</td>
<td>6</td>
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</tr>
<tr>
<td>Superior cervical ganglion</td>
<td>16</td>
<td>14</td>
<td></td>
</tr>
<tr>
<td>Sciatic</td>
<td>3.5</td>
<td>4</td>
<td>2.5</td>
</tr>
<tr>
<td>Phrenic</td>
<td>1.8</td>
<td>1.5</td>
<td>1.4</td>
</tr>
<tr>
<td>Superior mesenteric plexus</td>
<td>1.5</td>
<td>2</td>
<td></td>
</tr>
<tr>
<td>Thoracic sympathetic chain (including the ganglia)</td>
<td>14</td>
<td>12</td>
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</tr>
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</table>

superior cervical ganglion of the cat, dog and rabbit, by using bicarbonate-free Ringer containing physostigmine (1:50,000). The dialysates of the extracts were diluted and tested on the isolated frog heart. Table 1 gives the acetylcholine equivalent of extracts in γ per gram of nerve. A few experiments showing the inhibitory effects on the frog heart are illustrated in figure 1.

Control experiments were made with the other acetylcholine-extraction methods, namely, the acid-alcohol method (Engelhart, 1930), trichloracetic acid extraction (Chang and Gaddum, 1933), and the eserine-Ringer-heating method (Loewi and Hellauer, 1938 b); and the extracts were tested on the frog heart, the eserinized rectus abdominis of the frog (Chang and Gaddum, 1933), and on the longitudinal muscle of the leech (Minz, 1932). These comparative control titrations showed that the physostigmine-Ringer extract gave higher values than that resulting from either the alcohol or the heating procedure, and was about equal to the trichloracetic acid
extraction. By dialyzing the physostigmine-Ringer extract half the total acetylcholine content was excluded. This method has the advantage, however, of allowing tests for both positively and negatively acting substances. It was therefore used.

During the process of dialysis no acetylcholine or adrenaline was lost. Control experiments showed that when acetylcholine or adrenaline, diluted to 1:100,000,000 in Ringer’s solution containing physostigmine (1:50,000) and dialyzed against equal amounts of physostigmine-Ringer, exactly half the original acetylcholine or adrenaline concentration was found after 3 hours on both sides of the membrane. Such an experiment is illustrated in figure 2.

![Figure 1A](image)

**Fig. 1A. Normal frog heart.** In this and succeeding records the time signal marks 5-second intervals. Extracts were made with physostigmine-Ringer. At a, acetylcholine (a.ch.) 0.001γ; at b, a.ch. 0.005γ; at c, dialyzed extract (d.e.) of vagus of the cat; at d, a.ch. 0.01γ; at e, d.e. of cervical sympathetic nerve of the cat.

**B.** At a, d.e. of the sciatic of cat; at b, a.ch. 0.01γ; at c, d.e. of the cervical vago-sympathetic of dog; at d, a.ch. 0.01γ; at e, d.e. of the phrenic of cat; at f, d.e. of the thoracic chain and ganglia of cat; at g, a.ch. 0.008γ; at h, d.e. of mesenteric plexus of cat; at i, a.ch. 0.005γ.

**B. Extracts of postganglionic sympathetic fibers in physostigmine-Ringer solution.** In order to have pure postganglionic sympathetic fibers for extraction it was necessary to carry out some preliminary sterile operations on the animals used. Preganglionically denervated superior cervical ganglia (with the postganglionic fibers) of the cat and rabbit no longer contain acetylcholine one to two weeks after the operation. They contain an adrenaline-like substance having positive inotropic and chronotropic effects on the hypodynamic frog heart (fig. 3). The acetylcholine content of the superior cervical ganglion remained unaltered when the postganglionic fibers were cut.

In cats and dogs the vagi were cut below the diaphragm. Two weeks later, after complete degeneration of the vagus fibers, the postganglionic sympathetic fibers along the superior mesenteric artery were extracted and were found to contain no acetylcholine but an adrenaline-like substance
(fig. 3). Previous section of the sympathetic postganglionic fibers near the superior mesenteric ganglion, instead of the vagi, led to the disappearance of this substance from the isolated parts of the sympathetic fibers.

C. Extracts of different nerves in bicarbonate-free Ringer's solution. If extracts of mixed nerves were made with bicarbonate-free Ringer, without physostigmine, acetylcholine was totally destroyed and only the adrenaline-like substance was present. These experiments showed that an adrenaline-like substance can be extracted from all the nerves examined which contain postganglionic sympathetic fibers. The vagus fibers of the dog contain this substance; those of the cat do not contain it in sufficient
amount to be demonstrable. Evidence of the presence of the substance was found in extracts from the cervical sympathetic ganglia and their fibers, from the superior mesenteric plexus and ganglion and from the sciatic of the dog and cat. The substance was not found in the phrenic nerve. According to Verne (cf. Binet and Minz, 1936) the phrenics of the dog and cat are free of sympathetic fibers. It was also found in the superior mesenteric plexus of the sheep, cow and bull. Table 2 presents the adrenaline equivalent of extracts in γ per gram of nerve. A titration is shown in figure 4.

D. Some properties of the adrenaline-like substance extracted from the postganglionic sympathetic nerve fibers. As the extraction method shows, the adrenaline-like substance dialyzes easily through a cellophane membrane. If the test frog heart is washed with Ringer’s solution containing ergotoxine (1:100,000) until even high concentrations of adrenaline (1:10,000,000) are ineffective, the extract of the mesenteric plexus is likewise ineffective (fig. 5). If this extract is boiled for 2 to 3 minutes and tested after cooling it causes no change in the frog heart (fig. 5). Ashing the extract and subsequently making the ash up to the original volume with distilled water results also in a solution without a positive effect. If the solution is made alkaline and oxygen is bubbled through it for 5 hours, the adrenaline-like substance is destroyed. The substance, therefore, is of organic nature, oxydizable and thermolabile, and its action is abolished by ergotoxine.

**TABLE 2**

Adrenaline equivalent of extracts in γ per gram nerve

Average values calculated from 20 experiments. The maximal deviation from the average was 30 per cent.

<table>
<thead>
<tr>
<th>NERVES</th>
<th>CATS</th>
<th>DOGS</th>
<th>RABBITS</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cervical vagus</td>
<td>0</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>Cervical sympathetic trunk</td>
<td>0</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Superior cervical ganglia (with postganglionic fibers)</td>
<td>4</td>
<td>3.5</td>
<td></td>
</tr>
<tr>
<td>Sciatic</td>
<td>1</td>
<td>0.6</td>
<td>0.8</td>
</tr>
<tr>
<td>Phrenic</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Superior mesenteric plexus and ganglion</td>
<td>5</td>
<td>4.5</td>
<td></td>
</tr>
<tr>
<td>Thoracic sympathetic chain (including ganglia)</td>
<td>3</td>
<td>3</td>
<td></td>
</tr>
</tbody>
</table>

Discussion. The experiments reported in section A show, as has been reported by others, that the motor, parasympathetic and preganglionic sympathetic nerves and ganglia contain acetylcholine in different amounts.

Two to 4 weeks after section of the preganglionic fibers no detectable amount of acetylcholine was found in the superior cervical ganglia. Brown and Feldberg (1936) showed that the high acetylcholine content of the
normally innervated ganglion is dependent upon the integrity of the preganglionic fibers; normal ganglia yielded the high acetylcholine equivalent of 10 to 20\(\gamma\) per gram, which fell after denervation to 1 to 3\(\gamma\) per gram. Similarly MacIntosh (1938) examined the acetylcholine content of the preganglionically denervated ganglia and he found only 15 per cent of the normal acetylcholine equivalent present 72 hours after the operation. Finally Brücke (1937) found an excess of cholinesterase in the superior cervical ganglion of the cat, which esterase disappeared completely after section of the preganglionic fibers of the ganglion, even before these fibers could have completely degenerated. As shown in table 1 and in figure 1B, \(h\), extracts of the superior mesenteric plexus contain acetylcholine. That the presence of this substance is due to the mingling of cholinergic vagal fibers with adrenergic postganglionic sympathetic fibers is demonstrated in experiments reported in section B, figure 3C, \(a\) and \(b\), and D, \(a\) and \(b\). After complete degeneration of the vagal fibers, extract of the

Fig. 4A. Hypodynamic frog heart. Extracts made with bicarbonate-free Ringer. At \(a\), d.e. of cervical vagus of dog; at \(b\), d.e. of cervical vagus of cat; at \(c\), d.e. of phrenic of cat; at \(d\), d.e. of superior cervical ganglion of cat; at \(e\), adr. 0.01\(\gamma\); at \(f\), d.e. of superior mesenteric plexus and ganglion of cat; at \(g\), d.e. of cervical sympathetic nerve of cat; at \(h\), d.e. of sciatic of cat; at \(i\), d.e. of superior mesenteric plexus of dog.

B. At \(a\), adr. 0.01\(\gamma\); at \(b\), d.e. of superior mesenteric plexus of sheep; at \(c\), d.e. of cervical vagus of sheep; at \(d\), d.e. of cervical vagus of bull; at \(e\), d.e. of superior mesenteric plexus of cow; at \(f\), adr. 0.01\(\gamma\); at \(g\), d.e. of mesenteric plexus of bull; at \(h\), d.e. of superior mesenteric plexus of cow.
superior mesenteric plexus showed no signs of acetylcholine; only the adrenaline-like substance was manifest.

Since this research was completed Loewi and Hellauer (1938a, b) have shown that the preganglionic sympathetic fibers of cattle contain about six times more acetylcholine than the postganglionic fibers. The presence of acetylcholine in the postganglionic fibers might be explained as due to the admixture of some cholinergic elements. Euler and Gaddum (1931) showed that some cholinergic fibers are mixed with the postganglionic sympathetic fibers of the superior cervical ganglion of dogs.

The experiments outlined in section B present evidence that postganglionic sympathetic fibers contain no acetylcholine, but an adrenaline-like substance. The data from section C prove that an adrenaline-like substance can be extracted from all the nerves examined which contain postganglionic sympathetic fibers. The few preliminary experiments reported in section D lead to the inference that the properties of the substance extracted from adrenergic nerves are similar to those of adrenaline. The highest content was found in the superior mesenteric plexus, about 4 to 6γ per gram of nerve. This high amount of substance provides the possibility of studying its properties more completely in further experiments.

**SUMMARY**

1. By using bicarbonate-free Ringer’s solution containing physostigmine, extracts were made from different nerves of the cat, dog and rabbit. The extracts were dialyzed and tested on the frog heart. Extracts of
various nerves contain acetylcholine in different amounts (section A, fig. 1 and table 1).

2. Nerve trunks composed only of adrenergic fibers contain no acetylcholine but an adrenaline-like substance (section B, fig. 3).

3. From mixed nerves which contain postganglionic sympathetic fibers an adrenaline-like substance can be extracted (section C, fig. 4 and table 2).

4. The adrenaline-like substance is dialyzable, oxydizable and is destroyed by ashing or by boiling for a few minutes. It has a positive inotropic and chronotropic effect on a hypodynamic frog heart, and this effect is abolished by ergotoxine. The substance has properties similar to adrenaline (section D, fig. 5).

I wish to take this opportunity to express my appreciation to Prof. W. B. Cannon for his many attentions, both personal and scientific, during my stay in the Harvard Physiological Laboratory.

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