THEOPHYLLINE, EPINEPHRINE, AND NEOSTIGMINE FACILITATION OF NEUROMUSCULAR TRANSMISSION*

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Epinephrine can enhance transmission under certain conditions in both the central and peripheral portions of the nervous system. Increased quantities of acetylcholine are released in response to epinephrine at ganglionic and neuromuscular junctions.

At the biochemical level, epinephrine stimulates the formation of adenosine 3′,5′-phosphate (cyclic AMP) by preparations of many tissues, and in a few instances increased intracellular concentrations of cyclic AMP have been shown to result from the application of the hormone to tissue. Cyclic AMP levels also can be raised by theophylline, which inhibits hydrolysis of the nucleotide by a phosphodiesterase. These and other observations have led to the concept that cyclic AMP is the mediator of several epinephrine actions.

The possibility that the effects of epinephrine within the nervous system might also be mediated by cyclic AMP led to the present experiments. These have shown that theophylline is capable of potentiating the effects of epinephrine on sciatic-gastrocnemius preparations of the cat. This potentiation is blocked by propranolol, a β-adrenergic blocking agent, and does not occur in the curarized preparation.

Methods.—Cats of about 2 kg weight were anesthetized with ether, then injected intravenously with chloralose, 80 mg/kg; pentobarbital sodium, 6 mg/kg; and atropine sulphate, 1 mg/kg. The trachea was intubated and the adrenals tied off. Blood pressure was recorded by means of a mercury manometer connected to a carotid artery cannula. Supramaximal stimuli (1 msec duration, 6/min) were delivered without interruption from a Grass S4E stimulator through shielded electrodes to the distal portion of the divided sciatic nerve. A hole (3 mm diameter) was drilled through the lower end of the femur, which was then fixed by means of a steel rod. The Achilles tendon was freed and connected to a tension lever. Single shocks elicited an increase in tension of 1.5 to 2.0 kg. The muscle was stimulated directly in curarized preparations by stimuli (1 msec duration, 6/min) passed between the steel rod fixing the femur and a platinum wire in the Achilles tendon.

Amounts of L-epinephrine bitartrate and of neostigmine bromide which were given are recorded in terms of the salts. Values for theophylline (as a solution in warm 50 mM sodium acetate, pH 7.2, or as aminophylline) are expressed in terms of the base. Propranolol (Inderal) was obtained from Imperial Chemical Industries, and d-tubocurarine was kindly supplied by Burroughs, Wellcome and Co. All drugs were administered intravenously.

Results.—Neostigmine was used in these experiments because epinephrine consistently causes a greater increase in contractions of nerve-muscle preparations when an anticholinesterase is also present. Neostigmine was usually given three times at intervals of 20 minutes before an increase in tension was observed. There-
after, the same dose of neostigmine (20 or 30 µg) produced a small but reproducible increase in contractions. A single test solution containing epinephrine, neostigmine, and theophylline in various combinations was then injected. Subsequent test solutions were administered at intervals of not less than 30 minutes. Except where specifically noted, neostigmine was given before the combined injection and was included in each test mixture.

Theophylline regularly and strikingly potentiated the response to epinephrine and neostigmine (Fig. 1; Table 1, expts. 1-4). When neostigmine had not been given previously, epinephrine or epinephrine together with theophylline produced only a slight increase in tension (Table 1, expt. 5). Theophylline, which alone produced no effect on muscle contraction, increased the tension when administered with neostigmine (Table 1, expt. 6). Sympathetic fibers in the sciatic nerve as well as motor fibers were stimulated so that the effect may be due to potentiating the action of endogenous catecholamines.

Propranolol, a β-adrenergic blocking agent devoid of sympathomimetic activity, eliminated the theophylline effect when given before the combined injection of epinephrine, neostigmine, and theophylline (Table 2, expts. 7, 8). In a preparation to which neostigmine had been given previously, epinephrine increased the tension and propranolol blocked this effect also (Table 2, expt. 9).

If a preparation was fully curarized, and the muscle was stimulated directly, epinephrine and neostigmine failed to increase the tension and the theophylline effect was virtually abolished (Fig. 2). An identical result was obtained in a second preparation treated in the same way.

Discussion.—Theophylline potentiation of the increase in muscle tension caused
by epinephrine and neostigmine probably occurred at nerve endings, because in these experiments contractions of curarized, directly stimulated muscle were not increased. Since the phenomenon has notable parallels in other tissues in which cyclic AMP has been implicated, the following explanation of the present observations is attractive: epinephrine stimulates the formation of cyclic AMP within the motor nerve ending and the nucleotide then augments the release of acetylcholine at the terminal membrane. Repetitive firing of the muscle unit, secondary to an increase in the quantity and/or frequency of acetylcholine release, would result in an increased contractile response. Theophylline, by inhibiting the hydrolysis of cyclic AMP, would potentiate the effect of epinephrine in increasing the level of the nucleotide.

Phenomena closely analogous to that reported here, and which involve cyclic AMP, have been described for three other tissues. Epinephrine increases the intracellular concentration of cyclic AMP concurrently with contractile force of heart8, 9 and with fatty acid release from adipose tissue.10, 11 Theophylline potentiates both the rise in cyclic AMP and the fatty acid release from adipose tissue; it similarly potentiates the increase in cyclic AMP content and in water permeability due to vasopressin action on toad bladder.12, 13 β-Adrenergic blocking agents inhibit the increase in cyclic AMP and the associated physiological event in all three tissues.

The proposed role of cyclic AMP in augmenting the discharge of packets of acetylcholine at the axon terminal membrane has a close counterpart in the interpretation of experiments on amylase secretion from the parotid.14 Cyclic AMP is believed to be involved in the discharge of amylase granules because theophylline potentiates epinephrine-induced secretion of the enzyme, and dibutyryl cyclic AMP also stimulates amylase release. The nucleotide may play a comparable role in the release of insulin from islet cells provoked by theophylline or glucagon.15, 16

### TABLE 1

<table>
<thead>
<tr>
<th>Expt.</th>
<th>Epinephrine</th>
<th>Theophylline</th>
<th>Tension increase (kg)</th>
<th>Integral (kg X min)</th>
<th>Ratio:</th>
<th>+ Theophylline</th>
<th>0 Theophylline</th>
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<tr>
<td>1*</td>
<td>+</td>
<td>+</td>
<td>0.4</td>
<td>2.7</td>
<td>3.2</td>
<td>9.9</td>
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<tr>
<td>2</td>
<td>+</td>
<td>+</td>
<td>1.3</td>
<td>26.7</td>
<td>3.0</td>
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</tr>
<tr>
<td>3</td>
<td>+</td>
<td>+</td>
<td>0.8</td>
<td>5.9</td>
<td>2.6</td>
<td>4.3</td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>+</td>
<td>+</td>
<td>2.1</td>
<td>25.4</td>
<td>3.7</td>
<td>7.2</td>
<td></td>
</tr>
<tr>
<td>5†</td>
<td>+</td>
<td>+</td>
<td>0.4</td>
<td>3.2</td>
<td>4.8</td>
<td>6.9</td>
<td></td>
</tr>
<tr>
<td>6</td>
<td>0</td>
<td>0</td>
<td>1.5</td>
<td>23.0</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>0</td>
<td>+</td>
<td>0.5</td>
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<td>2.4</td>
<td>29.0</td>
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</table>

Mean: 3.6 7.1

The sciatic-gastrocnemius preparation, the conditions of stimulation, and the administration of drugs are described in the text. Amounts of drugs given were: epinephrine, 30 μg; neostigmine, 20 μg; theophylline, 100 mg. The tension increase recorded is the maximal value, and was attained 2 to 5 min after the injection of drugs. Because the duration of increased tension was also greater after theophylline than in the control, the over-all effect (Integral) was measured by tracing on paper the total area of increased tension and weighing the cutout trace.

* The amount of neostigmine and of epinephrine given was 30 μg.
† Neostigmine had not been given previously and was not included in the test mixture.
Repetitive firing of muscle units was probably the immediate basis of the increased contractions observed after administration of the various drug combinations in the present experiments. Neostigmine can increase the frequency of miniature end plate potentials and induce repetitive firing in motor nerve terminals quite apart from its well-known role as an acetylcholinesterase inhibitor. Epinephrine also is known to increase the frequency of miniature end plate potentials and to increase the amplitude of the end plate potential. If these effects were potentiated by theophylline and summed with those of neostigmine, multiple muscle spikes probably followed each stimulus to the nerve.

The suggested concept of cyclic AMP action in the motor nerve ending may have utility in considering the function of endogenous catecholamines in the central nervous system. Enzymes which catalyze the synthesis and the destruction of cyclic AMP occur at high levels of activity in the brain, and are relatively concentrated in the synaptosome fraction. Thus the enzymes which control cyclic AMP levels are strategically positioned to function in central cholinergic axons in the same way as proposed for peripheral nerve. Furthermore, the delayed onset and prolonged time course of catecholamine modulation of neuronal activity in brain would be compatible with the suggested role of cyclic AMP in mediating acetylcholine release.

Finally, the phenomenon which we have observed may pertain to therapy in myasthenia gravis. Ephetidrine is the one sympathomimetic agent which has established value in the treatment of patients with that disease. It is conceivable that theophylline or other cyclic phosphodiesterase inhibitors could potentiate the action of ephetidrine and thereby increase or prolong muscle strength.

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1 Bübring, E., and J. H. Burn, J. Physiol. (London), 100, 337 (1941).