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Effect of acetylcholine and vagostigmine on muscle and nerve of frog.

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Abstract

1) The effect of acetylcholine and vagostigmine on isolated muscle of frog and nerve-muscle preparations, with single induction shocks was studied. 2) Direct application of acetylcholine or vagostigmine to the muscle preparation produces a depressant action on the excitability as well as on the shortening height of the maximum contraction. The effects are more marked when the two drugs are applied simultaneously or successively than when applied separately. 3) Direct application of acetylcholine or vagostigmine to the nervous portion of the nerve-muscle preparation also produces a slight depressant action on the excitability of muscle to indirect stimulation applied to the nerve. 4) The applicability of the chemical transmission theory to amphibian muscles was discussed.

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If it be accepted that acetylcholine acts as a chemical transmitter at the motor nerve-endings (*Dale and Feldberg, 1934*), then if applied directly from the exterior or intravenously, this agent should elicit a similar muscular contraction in all animals. *Brown, Dale and Feldberg (1936)* proved experimentally that this is the case for mammalian muscle, but contradictory results have been obtained with amphibian muscle; *Brown* observed that with frog's muscle, as in mammalian muscle, acetylcholine caused muscular contraction and this was accompanied by typical action potentials. But, in our laboratory, *Yonezawa (1940)* using frogs prepared by the *Loewen-Trendelenburg's* method failed to observe muscular contraction in the hind-limbs after arterial injection of acetylcholine.

Another obstacle to the chemical transmission theory is that there is no evidence of eserine potentiation of the muscular response in amphibia (*Kruta, 1935; Cowan, 1936; Brown, 1937*). *Feng (1937)* was able to secure eserine potentiation by single shocks in the isolated nervesartorius preparation. *Hodes and Steiman (1939)* recently reported, (in their experiment on the effect of eserine, curare and acetylcholine on the normally circulated frog's gastrocnemius (*Rana catesbeiana*) subjected to varying frequencies of indirect stimulation) that the lymph-sac injection of eserine produces a single twitch potentiation and that after eserine, slow frequencies produce responses similar to those produced by higher frequencies in the normal muscle. Acetylcholine, they reported, arterially injected depresses the muscular contraction.

In order to investigate the action of acetylcholine and eserine on the amphibian neuromuscular system in a more simplified condition, the present investigation was undertaken using the isolated nerve-

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muscle preparation of the frog (*Rana nigromaculata*).

Methods and results.

Isolated sartorius and sciatic-gastrocnemius preparations were made, and immersed in *Ringer's* solution for 30 minutes before being used for the experiment. Values were determined for the minimum liminal strength of the stimulus to induction shocks (sciatic-gastrocnemius and sartorius preparations) and for the maximum strength of stimulus as well as for the shortening height at such stimulation strength (sartorius) both before and after the application of acetylcholine and vagostigmine of relative higher concentrations.

(1) *Experiments on sartorius muscle preparation* (see Tables 1, 2, 3).

As shown in Tables 1, 2 and 3, the values of the minimum liminal

Table 1. Effect of vagostigmine on threshold value, maximum strength of stimulus, height of contraction of sartorius muscle of frog.

No. of Exp.	Time of immers. (min.)	Threshold (coil dist. mm)	Max. stimul. (coil dist. mm)	Height of max. contr. (mm)	decrease of height in per cent
1. {Ringer Vag. (1:10 ⁴)	30	156 145	110 110	20 18	10
2. {Ringer Vag. (1:10 ⁴)	30	162 145	130 110	25 21	16
3. {Ringer Vag. (1:10 ⁴) " Ringer	10 30	137 133 120 130	90 10 90 10	44 29 18 28	34 59

Table 2. Effect of acetylcholine on threshold value, maximum stimulus, and height of max. contraction of sartorius muscle of frog.

No. of Exp.	Threshold (Coil dist. mm)	Max. stimul. (Coil dist. mm)	Height of max. contr. (mm)	decrease of height in (per cent)
1. {Ringer Ach. (1:10 ⁴) Ringer	120 86 120	90 70 70	46 35 37	24
2. {Ringer Ach. (1:10 ⁴)	114 115			
3. {Ringer Ach. (1:10 ⁴)	155 154	130 130	22 20	9
4. {Ringer Ach. (1:10 ⁴)	156 154	110 110	33 33	0

Table 3. Action of vagostigmine and acetylcholine on sartorius muscle of frog, when applied successively.

No. of Exp.	Threshold (Coil dist. mm)	Max. stimul. (Coil dist. mm)	Height of max. cont. (mm)	decrease of height (per cent)
1. { Ringer	136	120	27	
{ Vag. (1:10 ⁴)	126	100	13	52
{ Ach. (1:10 ⁴)	100	90	6	78
2. { Ringer	117			
{ Vag. (1:10 ⁴)	116			
{ Ach. (1:10 ⁴)	87			
3. { Ringer	143	120	24	
{ Vag. (1:10 ⁴)	140	110	25	
{ Ach. (1:10 ⁴)	64	0	3	87
4. { Ringer	153	130	22	
{ Vag. (1:10 ⁴)	140	130	21	5
{ Ach. (1:10 ⁴)	87	0	10	55

strength of stimulus needed to produce muscular contraction are increased by the application of both acetylcholine and vagostigmine separately. The effect of these drugs is more pronounced when both medicaments are applied simultaneously or successively. The value of the greatest strength of stimulus is also slightly increased by the application of these drugs separately, but is markedly increased by their successive application. In the height of the greatest shortening a marked decrease is also observed when these drugs are used. With regard to the effect of the two medicaments that of vagostigmine is generally superior in all respects to that of acetylcholine.

(2) *Experiments on sciatic-gastrocnemius preparation* (see Tables 4 and 5).

In the preceding experiments acetylcholine and vagostigmine were applied directly to the muscle. In the following experiments the effect was observed when the drugs were applied to the nerve. The nerve-muscle preparation was immersed in *Ringer's* solution, the nervous and muscular portions being separated by a paraffine wall. Induction shocks were applied indirectly to the nervous portion with *Lucas' fluid* electrodes, and the minimum liminal strength of the stimulus required to produce muscular contractions was determined. The results are summarized in the following tables.

When acetylcholine or vagostigmine are applied directly to the nerve, there is a slight increase in the value of the liminal strength of stimulus to indirect stimulation, but it is not so pronounced as in the case of the direct application of the drugs to the muscle.

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Table 4. Effect of acetylcholine on sciatic-gastrocnemius preparation of frog.

No of Exp.	Time of immers. (min.)	Threshold strength (Coil dist. mm)
1. { Ringer		186
{ Ach. (1 : 10 ⁴)	10	179
"	20	188
2. { Ringer		111
{ Ach. (1 : 10 ⁴)	3	89
"	5	89
3. { Ringer		144
{ Ach. (1 : 10 ⁴)	5	141
"	10	141
"	20	137
"	30	138
4. { Ringer		117
{ Ach. (1 : 10 ⁴)	3	112
"	7	112
"	12	112
5. { Ringer		148
{ Ach. (1 : 10 ⁴)	1	147
"	10	147
6. { Ringer		168
{ Ach. (1 : 10 ⁴)	2	161
"	10	161

Table 5. Effect of vagostigmine and acetylcholine on sciatic-gastrocnemius preparation of frog, when applied successively.

No. of Exp.	Time of immers. (min.)	Liminal strength (Coil dist. mm)
1. { Ringer		109
{ Vag. (1 : 10 ⁴)	10	106
"	30	107
{ Ach. (1 : 10 ⁴)	10	105
"	30	0 (dead)
2. { Ringer		189
{ Vag. (1 : 10 ⁴)	10	189
{ Ach. (1 : 10 ⁴)	10	184
3. { Ringer		126
{ Vag. (1 : 10 ⁴)	10	128
{ Ach. (1 : 10 ⁴)	3	129
"	15	129
"	30	127

No. of Exp.	Time of immers. (min.)	Liminal strength (Coil dist. mfm)
4. { Ringer Vag. (1 : 10 ⁴) Ach. (1 : 10 ⁴) " " " "	15	188
	3	181
	10	179
	30	180
		177
5. { Ringer Vag. (1 : 10 ⁴) Ach. " (1 : 10 ⁴) " "	2	162
	10	164
	2	163
		163
6. { Ringer Vag. (1 : 10 ⁴) Ach. (1 : 10 ⁴) " "	10	199
	1	197
	10	197
		198

Discussion.

Feng (1937) and *Cowan* (1938) reported eserine potentiation to single stimulation in the isolated frog's muscle. But *Brown* (1937), *Hodes* and *Steiman* (1939) have failed to secure positive results. My own results coincide with the results of the latter observers. There invariably occurs a depression of greater or minor degree of excitability as well as of the maximum muscular contraction height, when vagostigmine or acetylcholine are applied either separately or successively. No signs of the acetylcholine or vagostigmine potentiation were found. *Yonezawa* (1940) found no definitive effect of acetylcholine and vagostigmine of low concentration on sartorius muscle of frog, so I used these drugs in relatively high concentration, but found only the above described depressant effect. In the case of the application of these drugs to the nerve fibre, the tendency of the working mechanisms of these drugs is the same as in the muscle. From these results, we are not justified in concluding that the chemical transmission theory of nervous impulses applies to the neuromuscular mechanisms of amphibia.

Summary.

1) The effect of acetylcholine and vagostigmine on isolated muscle of frog and nerve-muscle preparations, with single induction shocks was studied.

2) Direct application of acetylcholine or vagostigmine to the muscle preparation produces a depressant action on the excitability as well as on the shortening height of the maximum contraction.

The effects are more marked when the two drugs are applied simultaneously or successively than when applied separately.

3) Direct application of acetylcholine or vagostigmine to the nervous portion of the nerve-muscle preparation also produces a slight depressant action on the excitability of muscle to indirect stimulation applied to the nerve.

4) The applicability of the chemical transmission theory to amphibian muscles was discussed.

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