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Decomposition Products of Nucleoproteins and Related Substances and Muscle Sensitivity to Acetylcholine and Potassium.*

CLARA TORDA AND HAROLD G. WOLFF.

From the New York Hospital and the Department of Medicine (Neurology) and Psychiatry, Cornell University Medical College, New York, N.Y.

In the search for substances with curare-like effect and substances that increase the sensitivity of the effector cells to acetylcholine the effect of decomposition products of nucleoproteins and related substances on the sensitivity of striated muscle to acetylcholine and potassium were investigated.

Nucleic acid and some decomposition products thereof (adenylic acid, guanylic acid, cytidylic acid, inosinic acid, adenosine, adenine, guanine, guanosine, inosine) and methylxanthines (caffein, theobromine, theofilline) may induce a vasodilatation,[1-11] may modify the contraction of the heart, striated and smooth muscle,[1,12-17] and methylxanthines and guanidine may induce a contracture of the striated muscle.[18] It is likely that these effects are independent of the action of acetylcholine-like substances liberated at the synapses since the above substances exert their effect even after administration of atropin.

Method. The rectus abdominis muscle of frog was excised and suspended in a muscle chamber containing 10 cc of Ringer's solution. The Ringer's solution was changed to an acetylcholine solution (50 μg acetylcholine per 100 cc Ringer's solution) for 2 minutes. The muscle was then washed with Ringer's solution for 10 minutes. This procedure was repeated until 3 successive exposures to the solution of acetylcholine gave similar responses. Afterwards the muscle was washed only for 5 minutes and immersed in one of the solutions of the substances used. A series of solutions containing each substance in increasing concentrations was used. The pH of all solutions used was corrected to 7. The height of contraction of the muscle was registered by an isotonic lever on a kymograph. The tracings were measured, the amount of contraction of the rectus abdominis muscle before application of the substances was taken as 100% and the amount of contraction after application of the substances was expressed as percent of it.

In another series of experiments a 20 mM solution of potassium chloride in Ringer's solution was used instead of acetylcholine.

Results. The effects of the substances used on the height of contraction of the rectus abdominis muscle induced by acetylcholine and potassium are given in Table I. Within

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TABLE I.

Effect of Substances on the Contraction of the Rectus Abdominis Muscle Induced by Acetylcholine or Potassium.

	Magnitude of contraction in % of control.* Contraction induced with:									
	Acetylcholine					Potassium				
	Concentration of the substances used (mg per 100 cc Ringer's solution):									
Substance	200	20	2	0.2	0.02	200	20	2	0.2	0.02
Nucleic acid (yeast)		82	80	100	102		75	78	80	78
Adenylic acid	105	102	102	102	100	171	143	138	125	105
Adenosine		99	98	98	102		135	125	112	113
Adenine sulfate		109	107	105	100		138	117	109	106
Adenine acetate		102	102	102	99		97	116	110	102
Guanine		107	110	104	101		228	185	161	146
Xanthine	113	114	105	106	101	175	162	152	148	127
Uracil	138	105	103	98	97	209	182	162	167	135
Thiouracil	175	106	105	101	97	258	170	140	126	113
Uric acid	105	102	99	105	105	224	150	150	142	124
Caffeine		299	123	96	95		281	157	124	122
Theobromine		182	107	93	93		253	160	118	114
Theofilline		152	102	98	104		348	156	128	110
Pyridine		151	101	100	100		180	133	118	115
2-methyl-5-ethoxymethyl-6-amino pyrimidine	340	136	105	100	98		172	127	107	111
4-methyl-5-β-hydroxyethyl thiazole	336	137	100	100	100		170	122	120	109
Pyrol		142	93	86	89		305	197	132	125
Piperidine		71	98	95	100		88	100	107	110
Alloxan	80	92	93	98	104	47	104	103	103	102
Methylguanidine		118	105	103	101		267	120	113	117
Urea	82	100	99	103	104	102	146	137	129	128
Thiourea	84	89	90	90	98	89	153	126	132	112

* Each value represents the average of 10 separate experiments. The S.E. of the mean for each value was less than $\pm 5\%$.

the range of concentrations used the effect of acetylcholine was either not modified (uric acid, adenosine, adenine, guanine, adenylic acid), or was not modified in low concentrations and was increased in higher (methylxanthines, uracil, thiouracil, methylguanidine, xanthine, pyridine, pyrol, 2-methyl-5-ethoxymethyl-6-amino pyrimidine, 4-methyl-5-β-hydroxyethyl thiazole), or was not modified in low concentrations and decreased in higher (alloxan, nucleic acid, urea, thiourea, piperidine).

The effect of potassium was either increased (uric acid, methylxanthines, uracil, thiouracil, urea, thiourea, adenosine, adenine, adenylic acid, guanine, xanthine, methylguanidine, pyridine, pyrol, 2-methyl-5-ethoxymethyl-6-amino pyrimidine, 4-methyl-5-β-hydroxyethyl thiazole) or decreased (nucleic acid, alloxan, piperidine).

The amount of contraction induced by potassium cannot be compared to that induced by acetylcholine since these substances cause different physicochemical changes in the muscle cells.[18,19] However, substances changing the amount of contraction induced by acetylcholine usually have similar effect on the muscle contraction following stimulation of the motor nerve and usually modify the activity of choline esterase. Substances changing the height of contraction induced by potassium usually modify the effect of other chemical agents that are able to induce muscle contraction by direct contact with the striated muscle.

Summary and Conclusions. 1. The effect of decomposition products of nucleoproteins and related substances on the contraction of the rectus abdominis muscle induced by acetylcholine and potassium was studied. 2. Adenosine, adenine, adenylic acid, and uric acid did not modify the effect of acetylcholine. 3. Xanthine, methylxanthines, uracil, thiouracil, pyridine, 2-methyl-5-ethoxymethyl-6-amino pyrimidine, 4-methyl-5-β-hydroxyethyl thiazole, methylguanidine, and pyrol did not modify the

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effect of acetylcholine when used in low concentrations. They increased the effect of acetylcholine when present in higher concentrations. 4. Nucleic acid, alloxan, urea, thiourea, and piperidine decreased the effect of acetylcholine when used in higher concentrations. 5. Adenosine, adenine, guanine, xanthine, adenylic acid, uric acid, methylxanthines, uracil, thiouracil, pyridine, 2-methyl-5-ethoxymethyl-6-amino pyrimidine, 4-methyl-5-β-hydroxyethyl thiazole, thiourea, urea, methylguanidine, and pyrol increased the effect of potassium in inducing muscle contraction. 6. Nucleic acid, alloxan, and piperidine decreased the effect of potassium in inducing muscle contraction.

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Effect of Alloxan on Carbohydrate and Uric Acid Metabolism of the Pigeon.*

MARTIN G. GOLDNER AND GEORGE GOMORI. With the technical assistance of Charlotte Stevens.

From the Department of Medicine, University of Chicago, Chicago, Ill.

Alloxan in a dose of 150 to 200 mg per kg injected intravenously into a pigeon causes an elevation of the blood sugar from the normal level of about 150 mg% to values between 300 and 400 mg% within 24 to 48 hours. One day after the injection the animals appear very sick; they are weak and drowsy, sit with their eyes closed, make no attempt to move or fly and usually die in this stupor within 2 or 3 days. Smaller doses of alloxan (60-125 mg per kg) may cause a similar syndrome except that the blood sugar may remain within the normal range. A disturbed carbohydrate metabolism can therefore not be the only cause of this condition.

At autopsy such pigeons present a very unusual picture. All serous membranes, especially the pericardium, the pleura, and the surface of the liver are covered with a thin whitish layer which at first glance may look very much like fibrin as seen in polyserositis. This material, however, can easily be removed from the membranes, and identified chemically and microscopically as sodium urate. In one instance 8 mg of sodium urate were collected from the pericardium and the liver surface alone.

Histological examination of the kidneys reveals massive infiltration with sodium urate which is deposited mainly in the greatly dilated tubules, but also in the interstitial tissue. Smaller collections of crystals are found on the serosal surfaces and occasionally in the parenchyma of the liver. Evaluation of the changes in the pancreatic islets is very difficult because even the normal pigeon pancreas may contain extremely few beta cells. However, in some cases which showed hyperglycemia no beta cells could be found at all, or the occasional cells found looked profoundly degranulated. In other cases the islets were normal.

In view of these findings, it seemed of importance to study the effect of alloxan on the uric acid metabolism of pigeons. A series of more than 30 pigeons was injected with various doses of alloxan, and the blood uric acid level was determined before and at intervals after the injection. Folin's[1] method for the estimation of uric acid was used. Blood samples were taken from the wing vein. The normal blood uric acid content of our pigeons

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