16654

Action of Acetylcholine, Histamine and Cholinesterase Inhibitors on Isolated Ileum in Relation to its Survival Time.*

PETER FLESCH AND ERNEST KUN. (Introduced by E. M. K. Geiling.)

From the Department of Pharmacology, University of Chicago.

In preliminary experiments it was found that the atropinized ileum of the guinea pig began to react to acetylcholine 2½ hours after the death of the animal. Observations of this nature were made by Le Heux,[1] who showed that the rhythmic peristaltic contractions of the isolated guinea pig ileum could no longer be blocked by atropine after the intestine had been kept in Locke solution for several hours; he also noticed a similar behavior in the isolated small intestine of the rabbit. We therefore decided to study the correlation between the time elapsed after death and the responses to acetylcholine, histamine and various cholinesterase inhibitors of the atropinized and non-atropinized intestinal strips of several species of animals.

Experimental. Guinea pigs, rabbits, rats and chickens were used. Strips of ileum were suspended in a Tyrode bath of 45 ml at 37°C. The temperature of the bath for the chicken intestine was kept at 41°C. The experiments were started within 15 minutes after death.

At the beginning of each experiment, several successive doses of 5 to 10 μg acetylcholine bromide were added to the bath. At the height of the second contraction caused by acetylcholine (one minute after addition of the drug), histamine phosphate was added to the bath. After each contraction, the Tyrode solution was changed and a resting period of 3 minutes was allowed.

Acetylcholine bromide and histamine phosphate were added in amounts which caused submaximal contractions. Satisfactory results were obtained by adding the following doses, expressed in μg, to a bath of 45 ml volume:

	Guinea pig	Rat	Rabbit	Chicken
Acetylcholine bromide	0.2-0.5	0.2-0.6	0.2-0.6	0.05-0.3
Histamine phosphate	0.5-1.0	60. -150	10. -40	5. -10

These doses correspond to those recommended by other authors.[2-6]

The cholinesterase inhibitors were used in the following amounts: Physostigmine sulfate: 0.5-10 μg; neostigmine methylsulfate: 1-10 μg; hexaethyl tetraphosphate: 10 μg.

When the intestine was treated with both acetylcholine and histamine, the second drug was added only after the first contraction had reached its maximum. The same precaution was observed when histamine was followed by acetylcholine.

In one series of experiments the intestinal strips were suspended in atropinized Tyrode solution. The intestine was considered to be completely atropinized when it failed to respond to repeated additions of 10-50 μg acetylcholine bromide. For this purpose the following concentrations of atropine were found to be satisfactory: 1 mg atropine sulfate per liter Tyrode for the guinea pig, 2 mg per liter for the rat and 3 mg per liter for the chicken and rabbit. Histamine was used in the same amounts as in the case of non-atropinized intestines.

In all experiments control strips from the same animal were kept in regular and atropinized Tyrode solution for several hours at room temperature or in a bath at 37°C. When

* This work was aided by a grant from the Office of Naval Research N6ori-20, Task Order 11.

1 Le Heux, J. W., *Arch. f. d. ges. Physiol.*, 1920, **179**, 177.

2 Levy, J., and Michel, Z., *J. Physiol. et Pharm. gén.*, 1937, **35**, 389.

3 Kahlson, G., *Arch. f. exp. Path. u. Pharmakol.*, 1934, **175**, 189.

4 Page, I. H., and Schmide, E., *Z. f. Physiol. Chem.*, 1930, **191**, 262.

5 Ambache, N., *J. Physiol.*, 1946, **104**, 266.

6 Erspamer, V., *Arch. f. exp. Path. u. Pharmakol.*, 1940, **196**, 343.

the piece of ileum which was subjected to frequent stimulation began to show marked and reproducible changes in its reaction to histamine, it was replaced by a control strip. Then, by registering the contractions, it was observed whether the control strip, which had not been stimulated before, showed the same types of response.

Results. In the guinea pig ileum, in non-atropinized media, the contractions caused by acetylcholine and by histamine mutually reinforced each other during the first hour after death. (Fig. 1, 2). Toward the end of the third hour when histamine was added to the bath, after the intestine had been contracted by acetylcholine, there was a small initial contraction immediately followed by a pro-

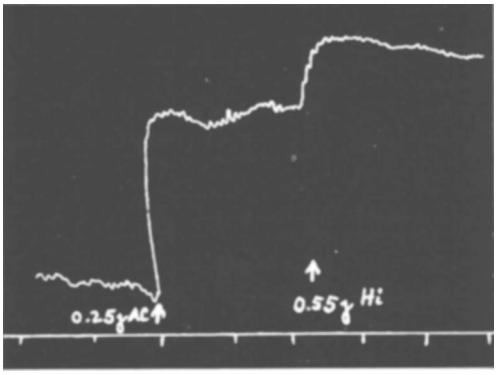

FIG. 1.
Effect of acetylcholine and histamine on the isolated guinea pig ileum ½ hour after death of the animal. Consecutive contractions are additive. Time in 30 second intervals.

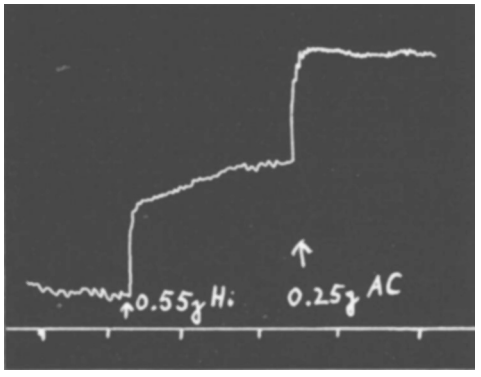

FIG. 2.
Effect of histamine and acetylcholine on the isolated guinea pig ileum ½ hour after death of the animal. Time in 30 second intervals.

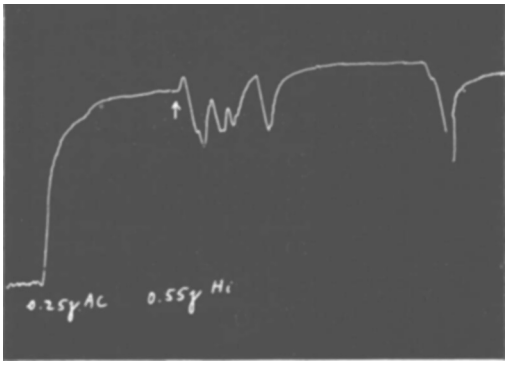

FIG. 3.
Effect of acetylcholine and histamine on the isolated guinea pig ileum 3½ hours after death of the animal. Histamine causes relaxation when added after acetylcholine.

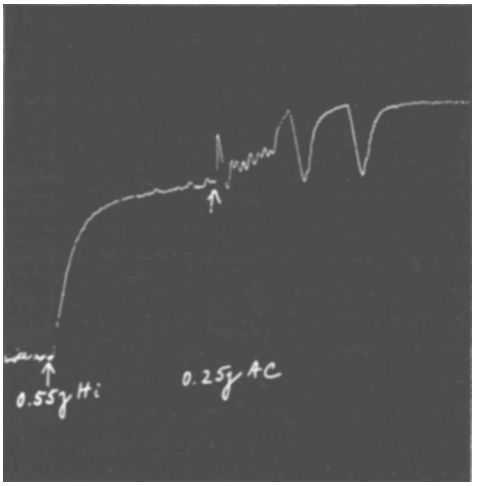

FIG. 4.
Effect of histamine and acetylcholine on the isolated guinea pig ileum 3½ hours after death of the animal. Acetylcholine reinforces the contraction caused by histamine.

found relaxation. The muscle then recovered and gradually reached the level of contraction attained prior to the addition of histamine. (Fig. 3.) The situation was different when, at the height of a histamine contraction, acetylcholine was added to the bath. Acetylcholine always reinforced the histamine contraction. At the beginning of the experiment simple summation occurred. However, toward the end of the third hour, the contraction initiated by the addition of acetylcholine was interrupted by a series of large peristaltic waves. These in-

TABLE I.
Sensitivity of the Isolated Guinea Pig Ileum to Acetylcholine at Different Intervals After Death.

Time after death in min.	Acetylcholine to 45 ml bath fluid μg	Height of contraction in mm
10	10	42
15	10	72
20	10	96
25	10	96
30	10	87
At 35 min. atropinized Tyrode was added (1 mg atropine sulfate/liter)		
40	10	0
90	10	0
120	10	0
135	10	0
140	10	3
150	10	9
180	10	18
195	10	15

creased in magnitude and frequency as the experiment proceeded. Eventually the muscle reached a plateau which was invariably higher than the one which it maintained after the first addition of histamine. (Fig. 4) Control muscles kept in Tyrode solution for several hours before the addition of histamine reacted in the same way as those which had undergone frequent stimulation.

In the rabbit ileum histamine and acetylcholine mutually reinforced each other during the first 2 hours after death. From the third or fourth hour on, a summation occurred only when histamine was followed by acetylcholine, but not when acetylcholine was followed by histamine.

During the first hour after death histamine elicited contraction of the rat intestine. As early as 2 hours after death the same dose of histamine caused relaxation or provoked no response. In the chicken intestine, 3 hours after death, the reaction to histamine became reversed at a time when the intestine was still responding with the usual contraction to the addition of acetylcholine.

The contractions caused by the cholinesterase inhibitors had the following characteristics: 1. The response was delayed while the response to histamine was always immediate. The delay between the addition of the drug and the contraction was most marked in the guinea pig. In all 4 species the time interval between the addition of the drug and the contraction was longest with physostigmine, least pronounced with neostigmine. 2. The response was gradual, *i.e.*, it took several minutes before the muscle reached the height of its contraction. Within the range of doses used, the delay in the response and the amplitude of the contraction were largely independent of the dose administered. 3. The intestinal strips still reacted to acetylcholine and to the cholinesterase inhibitors with a contraction at a time when histamine provoked no more response or relaxation only.

A total of 12 experiments was carried out on rabbits, 20 on rats and 9 on chickens. Of these 9 rabbits, 18 rats and 6 chickens showed the above described phenomena. Without exception, the small intestines of the individuals which did not undergo these changes responded with unusually sluggish contractions both to acetylcholine and to histamine. In some instances such delayed responses could be ascribed to the fact that the animals were old, or that the intestines had been subjected to prolonged handling before stimulation. However, in the case of the guinea pig, in all 24 animals which were used during the winter months, the reaction of the intestine underwent the changes described above. In 8 experiments which were carried out in the spring on the same strain of guinea pigs, the intestine reacted in a sluggish way to acetyl-

choline and to histamine. A decreased sensitivity of the isolated guinea pig ileum to histamine in the springtime has been reported by Kwiatkowski.[7]

In all experiments, the atropinized ileum of all 4 species did not react to acetylcholine and to the cholinesterase inhibitors during the first 2 to 3 hours after death. At the time when the non-atropinized intestine began to fail to contract from histamine, the responses of the atropinized muscles to acetylcholine and to cholinesterase inhibitors were no longer blocked by atropine in the same concentration which was sufficient to inhibit the action of acetylcholine immediately after death. A typical experiment is shown in Table I.

Discussion. From our experiments it seems that several hours after death a certain change occurs in the intestinal muscle. This change in the surviving tissue results in a reversal of the response to histamine at a time when the muscle still contracts to acetylcholine and to the cholinesterase inhibitors. These observations do not support the recent theory[5] that histamine contracts the smooth muscle by causing a liberation of acetylcholine. The different character of the contractions caused by histamine and by the cholinesterase inhibitors and the different reactions to atropine are also against the theory that histamine stimulates the smooth muscle of the intestine through a cholinergic mechanism. On the other hand, the possibility cannot be excluded that there is "an interdependence between the sites of attack" of histamine and acetylcholine as was suggested by Rocha e Silva.[8] It is also obvious that when experiments are carried out with isolated intestinal muscle, it is necessary to take into account the time which has elapsed since the death of the animal, particularly when histaminic responses and the reactions of the atropinized muscles to parasympathetic stimulants are being observed.

Summary. The action of acetylcholine, histamine, physostigmine, neostigmine, and hexaethyltetraphosphate was studied on the isolated ileum of the guinea pig, rabbit, rat and chicken in non-atropinized and atropinized media. In all 4 species the contractions caused by acetylcholine and histamine reinforced each other immediately after the death of the animal, regardless of the order in which they were added to the bath. Two and a half hours to 3 hours after death, histamine failed to contract the intestine when it was added at the height of an acetylcholine contraction. In the rat, rabbit and chicken the intestine no longer responded to histamine at a time when it still reacted to acetylcholine and to the cholinesterase inhibitors. In all 4 species atropine failed to block the action of acetylcholine and cholinesterase inhibitors in intestinal strips which were at least 3 hours old.

[7] Kwiatkowski, H., *J. Physiol.*, 1943, **102**, 32.

[8] Rocha e Silva, M., *J. Pharm. Exp. Ther.*, 1944, **80**, 399.