

PHARMACOLOGY

EFFECT OF BILE AND ITS MAIN COMPONENTS ON THE MUSCARINE-LIKE CHOLINERGIC SYSTEMS OF THE SMOOTH MUSCLES

Ya. V. Ganitkevich

UDC 612.73.014.46:612.822.2]-06:612.357

The object of this investigation was to study the effect of small concentrations of bile and of its components on the sensitivity of the smooth muscles to acetylcholine.

EXPERIMENTAL METHOD

Experiments were carried out on isolated organs of rabbits and frogs. The lungs, and rings of the stomach muscle of the frog and segments of the small intestine (mostly the duodenum, occasionally the jejunum) of the rabbit were used. The original contractions of these organs evoked by acetylcholine (10^{-7} - 10^{-5} g/ml) and their contractions after the addition of bile to the Ringer's solution (in the case of the rabbit's intestine to the Tyrode solution) and, in some experiments, the contractions after rinsing out the bile were recorded for 1 min by the usual methods. The action of ox bile, of bile acids (glyco- and taurocholic acids) isolated from the bile by the usual method [1], and of chemically pure bilirubin (acid form, $C_{33}H_{36}N_4O_6$) in concentrations close to the blood levels of these substances were studied. Altogether 300 experiments were carried out. The numerical data were analyzed by statistical methods.

EXPERIMENTAL RESULTS

After administration (for 5 min) of ox bile solutions in dilutions of 1:100-1:500,000, in all the groups of experiments the reaction to acetylcholine was increased (by comparison with the control tests). Solutions of the bile acids also increased the contractions of the lung muscles produced by acetylcholine, although in higher concentrations (10-50 mg %) their action was noticeably weaker than that of the corresponding bile solutions.

In some experiments bile acids and acetylcholine were administered together. The results of these experiments were similar to those obtained during the prolonged (5 min) action of the bile acids.

It was found that bilirubin, in a high concentration (10 mg %) caused a marked decrease in the amplitude of the acetylcholine contraction; in a smaller concentration (1 mg %) it produced no significant changes.

It may be concluded from the results obtained previously, indicating that solutions of bile and bile acids following administration have no effect on the activity of acetylcholine itself or of cholinesterase [2], that solutions of bile and bile acids in small concentrations considerably increased the excitability of the muscarine-like cholinergic systems of the smooth muscles of the lungs. This action of bile was mainly due to the effect of the bile acids which it contained. Bilirubin, in a comparatively high concentration (10 mg %) may depress the excitability of the muscarine-like cholinergic systems.

It is clear from the results obtained with the rings of the pyloric portion of the stomach that relatively high concentrations of bile (dilution 1:100) sharply depress the acetylcholine contraction, whereas under the influence of low concentrations the amplitude of the contractions increase (Table 2).

Bile acids in high concentrations (50 mg %) also depressed the reactions to acetylcholine. During the action of smaller concentrations (1-10 mg %) both an increase and a decrease in the amplitude of the contractions was observed; these changes are not statistically significant.

Bilirubin, in a high concentration (10 mg %) caused a clear increase in the reaction of the muscles of the pyloric portion of the stomach to acetylcholine, while in a lower concentration (1 mg %) it was inactive.

Observations on the rings of muscle from the cardiac portion of the stomach showed that the addition of bile in such cases caused a relaxation, and in others an intensification of the acetylcholine contractions. These changes

Department of Normal Physiology, Ivano-Frankovsk Medical Institute (Presented by Active Member of the Academy of Medical Sciences of the USSR V. V. Parin). Translated from *Byulleten' Éksperimental'noi Biologii i Meditsiny*, Vol. 62, No. 9, pp. 66-69, September, 1966. Original article submitted June 6, 1964.

TABLE 1. Effect of Solutions of Bile, Bile Acids, and Bilirubin on Amplitude of Acetylcholine Contraction of the Smooth Muscles of the Frog's Lung

Acting solution	Concentration of bile acids in solution (in mg %)	Amplitude of muscle contractions (in percent of initial contraction)	
		Limits of variations	M ± m
Repeated control tests		100-121	107 ± 6
Bile:			
1:100	50	142-192	167 ± 7
1:500	10	171-209	177 ± 8
1:5000	1	108-140	127 ± 5
1:50,000	0.1	122-188	149 ± 9
1:500,000	0.01	100-171	121 ± 8
1:5,000,000	0.001	91-127	111 ± 7
Bile acids	50	129-172	146 ± 8
"	10	119-173	143 ± 9
"	1	111-169	131 ± 9
"	0.1	120-166	142 ± 7
"	0.01	101-136	122 ± 5
Bile acids simultaneously with acetylcholine	50	127-200	167 ± 12
Bilirubin:			
10 Mg %		72-107	86 ± 4
1 Mg %		76-143	100 ± 7

TABLE 2. Effect of Solutions of Bile, Bile Acids, and Bilirubin on the Amplitude of Acetylcholine Contractions of the Muscles of the Pyloric Portion of the Stomach

Acting solution	Concentration of bile acids in solution (in mg %)	Amplitude of muscle contractions (in percent of initial contraction)	
		Limits of variations	M ± m
Repeated control tests		74-114	95 ± 6
Bile:			
1:100	50	0-90	36 ± 11
1:500	10	80-165	112 ± 10
1:5,000	1	61-180	111 ± 12
1:50,000	0.1	76-188	128 ± 13
Bile acids	50	0-85	27 ± 11
" "	10	25-144	88 ± 21
" "	1	46-141	102 ± 10
Bilirubin:			
10 mg %		90-150	131 ± 9
1 mg %		78-116	98 ± 7

TABLE 3. Effect of Bile, Bile Acid, and Bilirubin on Amplitude of Acetylcholine Contractions of the Intestinal Muscles of the Rabbit

Acting solution	Concentration of bile acids in solution (in mg %)	Amplitude of muscle contractions (in percent of initial contraction)	
		Limits of variations	M ± m
Repeated control tests		89-115	103 ± 3
Bile:			
1:100	50	10-68	41 ± 4
1:500	10	69-138	108 ± 6
1:5000	1	98-140	118 ± 6
Bile acids	50	39-129	87 ± 8
" "	10	100-166	124 ± 6
" "	1	98-143	111 ± 5
Bilirubin:			
10 mg %		75-192	118 ± 4
1 mg %		81-130	104 ± 4

were not statistically significant, although during the action of low concentrations of bile (dilutions 1:500-1:5000) a tendency for the contractions to increase was observed. Similar results were obtained in experiments to test the action of solutions of bile acids, but in contrast to bile, these acids in a high concentration (50 mg %) caused a significant decrease in the amplitude of the contractions. When the action of bile was studied on isolated segments of the rabbit's intestine, together with changes in the sensitivity of the muscle to acetylcholine, changes were observed in the amplitude of the rhythmic contraction and the tone of the intestine. The addition of bile to the Tyrode solution in high concentrations (dilution 1:100-1:500) caused an immediate and sharp decrease in the rhythmic contractions of the intestine, or even their total disappearance, and a fall in its tone. Lower concentrations of bile produced no clear changes in the automatic activity and tone of the intestinal muscle.

Observations on the acetylcholine contractions of the intestine showed that only the highest of the concentrations of bile used (dilution 1:100) caused a sharp depression of the sensitivity of the muscles to acetylcholine (Table 3).

Lower concentrations of bile increased the reactions of the muscles to acetylcholine. The greatest increase in the amplitude of the acetylcholine contractions took place after addition of bile in a dilution of 1:5000.

The solutions of bile acids had the same effect as those of bile, although during the action of high concentrations (50 mg %) the depression of the reactions was less severe. The most marked increase in the sensitivity to acetylcholine was found when the concentration of bile acids was 10 mg %.

It was noted that bilirubin affected both the acetylcholine contractions and the rhythmic movements of the intestine. In a concentration of 10 mg %, in 9 of 15 experiments bilirubin caused a considerable increase in the amplitude of the rhythmic contractions, which differed significantly from the changes developing after the addition of the alkaline solvent only. Against the background of the action of bilirubin, a marked increase took place in the amplitude of the contractions in response to acetylcholine, and this was particularly marked in the experiment in which the amplitude of the rhythmic contraction was increased.

Rinsing out the bile, bile acids, and bilirubin in all the series of experiments led to a more or less complete restoration of the reactions of the muscles to acetylcholine to their initial level.

It may be concluded from the results that the disturbances of the autonomic functions during retention of bile in the body (bradycardia, hypotension, changes in the motor and secretory activity of the gastrointestinal tract, and so on) may be associated with changes in the sensitivity of the muscarine-like cholinergic systems to acetylcholine as a mediator of the autonomic nervous systems, resulting from the action of the components of the bile (bile acid, bilirubin). Meanwhile, changes in the excitability of the muscarine-like cholinergic systems may play an important role in the mechanism of action of bile on the functional state of the central nervous system.

LITERATURE CITED

1. S. D. Balakhovskii and I. S. Balakhovskii, *Methods of Chemical Analysis of Blood* [in Russian], Moscow (1953).
2. Ya. V. Ganitkevich, *Byul. éksp. biol.*, No. 8, 78 (1964).