THE EFFECT OF BILE AND ITS CHIEF CONSTITUENTS ON THE NICOTINE-LIKE CHOLINERGIC SYSTEMS OF SKELETAL MUSCLES

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Ya. V. Ganitkevich

Department of Normal Physiology (Head, Professor, V. S. Raitses),
Ivano-Frankovsk Medical Institute
(Presented by Active Member AMN SSSR, N. I. Grashchenkov)
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Experimental and clinical jaundice are accompanied by considerable changes in the activity of the nervous system. In previous investigations we studied the character of the changes in the processes of higher nervous activity during the experimental retention and loss of bile [3, 4]. It remains unexplained, however, which constituents of bile exert a neurotropic action, and on which elements of the nervous system they act. Although it is generally accepted that toxic effects during retention of bile are produced by the bile acids, there is no direct, convincing evidence in support of this view. Some authors consider that the cause of the observed disturbances is a weakening of the barrier function of the liver [6, 10] or a disturbance of ionic equilibrium [8], while others describe a toxic action of bilirubin on the cells and tissues [9, 14].

In order to investigate the mechanism of the neurotropic action of bile, we have studied the effect of bile and of its constituents in small concentrations, close to those found in the blood, on mediator systems. In this paper we present the results showing the effect of bile and its constituents on the nicotine-like cholinergic systems of the skeletal muscles.

EXPERIMENTAL METHOD

Experiments were conducted on the rectus abdominis muscle of the frog by the usual method. One or 2 recordings were made of the initial contractions of the muscle to acetylcholine chloride (10^{-6}) and of the contractions 5 min after the addition of bile solution and washing. In some experiments the muscle was preliminarily eserinized.

The action of solutions of ox bile, human bile (from the gall bladder, obtained during operation, and from the liver through a fistula), and dog's bile (from the gall bladder and liver). The concentration of bile acids in the bile was determined by the method of Scherer and Cooney and the bilirubin by Van den Bergh's method. The solutions used contained 50 mg% bile acids (equivalent to 1 mg% bilirubin) or less (down to minimal active concentrations). Bile acids obtained from ox bile by the usual method were used in the same concentrations [2]. In addition the action of chemically pure preparations of the sodium salts of cholic and glycocholic acids and also of bilirubin was investigated. Altogether 240 experiments were carried out. In each group of experiments the mean, the standard deviation and the standard error of the arithmetical mean were calculated.

EXPERIMENTAL RESULTS

Solutions of bile in concentrations close to that found in the internal milieu of the organism had a marked effect on the excitability of the cholinergic systems of the skeletal muscles (Table 1). Higher concentrations of bile sharply depressed the contractions of the muscles during the action of acetylcholine, whereas lower concentrations clearly increased the amplitude of the contractions. Control experiments confirmed that bile, when mixed with acetylcholine solution and left for 5-10 min, did not alter its activity. Under the influence of the concentrations of bile tested, the cholinesterase activity in vitro likewise was not noticeably changed. In experiments on eserinized

TABLE 1. Effect of Solutions of Ox Bile on Amplitude of Contractions of Skeletal Muscle Under the Action of Acetylcholine

Dilution of bile	Conc. of bile acids in solution (in mg%)	Amplitude of contraction of muscle (as % of initial contraction)			
		limits of variation	M	σ±	m±
Repeated control tests:		82-108	93	9	3
1:100	50	27-44	37	4	2
1:500	10	43-86	61	11	5
1:5,000	1	74-110	95	6	2
1:50,000	0.1	88-127	110	8	4
1:500,000	0.01	100-123	109	8	3
1:1,000,000	0.005	91-119	102	12	5

TABLE 2. Effect of Solutions of Bile Acids and Bilirubin on Amplitude of Contractions of Skeletal Muscle Under the Action of Acetylcholine

Preparation	Concentration (in mg%)	Amplitude of contraction of muscle (as % of initial contraction)			
		limits of variation	M	σ±	m±
Repeated control tests		70-108	92	10	3
Bile acids from ox bile	50	31-58	41	7	3
The same	10	50-89	74	10	4
" "	1	86-114	99	6	2
" "	0.1	84-115	99	9	4
" "	0.01	88-120	98	11	4
Cholic acid	50	21-65	41	14	6
" "	10	53-93	80	12	5
" "	1	92-120	105	10	4
" "	0.1	79-127	94	15	6
Glycocholic acid	50	34-51	42	7	3
" "	10	44-100	74	20	8
" "	1	72-104	87	9	4
" "	0.1	76-100	91	5	2
Bilirubin	10	84-103	93	4	2

muscles and in those without the use of eserine the influence of bile was similar in character. Consequently, the difference in the amplitude of the muscular contractions in response to acetylcholine demonstrates that changes took place exclusively in the excitability of the muscle cholinergic systems.

Hence, under the influence of high concentrations of bile, the excitability of the nicotine-like cholinergic systems of the skeletal muscles fell sharply, while under the influence of lower concentrations it showed a clear increase.

Experiments in which the constituents of bile were used showed that bile acids act in a similar manner to bile solutions, whereas bilirubin had no significant effect on the excitability of the cholinergic systems (Table 2).

In a concentration of 50-10 mg%, all the bile acids tested depressed the excitability of the cholinergic systems to an equal degree, although their action was less marked than that of corresponding solutions of ox bile. These results are in agreement with the fact, reported in the literature, that the reaction of skeletal muscles to acetylcholine is depressed under the influence of high concentrations of bile acids [7, 13], and also with results showing that the addition of bile acids to perfusion fluid as a rule depresses the sensitivity of the cells of the cervical sympathetic ganglion to acetylcholine [12]. In lower concentrations (1, 0.1, 0.01 mg% only cholic acid and bile acids from ox bile caused a significant increase in the excitability of the cholinergic systems. Glycocholic acid had no such action.

TABLE 3. Effect of Solutions of Human Bile and Dog's Bile on Amplitude of Contractions of Skeletal Muscle Under the Action of Acetylcholine

Dilution of bile	Conc. of bile acids in solution (in mg%)	Amplitude of contraction of muscle (as % of initial contraction)			
		limits of variations	M	σ±	m±
Human bile					
1:100	50	26-85	56	24	8
1:1,000	5	70-112	87	12	5
1:10,000	0.5	76-92	87	7	3
1:100,000	0.05	72-118	94	10	4
1:1,000,000	0.005	90-122	102	12	5
1:10,000,000	0.0005	91-128	106	13	5
Dog's bile					
1:100	50	19-52	32	12	5
1:1,000	5	31-100	59	22	9
1:10,000	0.5	77-119	98	15	5
1:100,000	0.05	82-114	97	12	5
1:1,000,000	0.005	84-103	96	5	2
1:10,000,000	0.0005	70-135	95	20	8

It is clear from the results described above that the action of bile on the cholinergic systems is dependent on the concentration of bile acids in the bile and is not dependent on the presence of bilirubin. At the same time it should be noted that the depressant and, in particular, the stimulant action of the bile acids used were much weaker than the action of the bile solutions. It is evident that, besides cholic and glycocholic acids, other factors also present in bile solutions must possess neurotropic activity. The possibility is not ruled out that these may be other highly active bile acids, such as desoxycholic or lithocholic acids or their derivatives. This may afford an explanation of the "dissociation" observed in jaundice—i.e., the discrepancy between the severity of the disorder and the level of cholic acid in the blood.

Since bile from different species of animals differs considerably in its content of bile acids, we decided to compare the effects of human bile and dog's bile on the cholinergic systems. Comparison of the experimental results (Table 3) shows that human bile in high concentrations had a slightly less marked depressant action than ox bile and dog's bile. The increase in the excitability of the cholinergic systems during the action of low concentrations of human bile was slight but significant. Dog's bile in lower concentrations did not cause significant changes, although a consistent tendency towards an increase in excitability was observed.

These results show that the composition of bile is an important factor in determining the extent of its neurotropic action. In order to elucidate the action of bile demonstrated by the foregoing investigation, experiments were carried out in which the duration of action of the bile was varied and unithiol, a donor of sulfhydryl groups, was used.

During the action of low concentrations of bile, causing an increase in the strength of muscular contractions to acetylcholine, the same effect was observed when the bile acted for a long (5 min) or short (1 min) period, and even when the bile and acetylcholine acted at the same time.

However, the brief (1 min) action of bile or bile acids in a concentration of 50-10 mg% had only a very slight effect on the excitability of the cholinergic systems. Only with a longer duration of action (4-5 min) was the depressant effect of bile observed to its full extent. Washing of the muscle led to recovery of the excitability of the cholinergic systems. The addition of unithiol (0.1%) before acetylcholine fully or partly restored the excitability of the cholinergic systems, depressed by bile acids. Whereas bile acids in a concentration of 10 mg% lowered the amplitude of the contractions of the muscle from 93±5% to 74±4%, after the action of the same bile acids and addition of unithiol the amplitude of the contractions reached 94±7% of its initial value. When the concentration of bile acids was 5 mg%, unithiol increased the amplitude of the contractions from 41±3 to 62±4% of their initial value.

The increase in the excitability of the cholinergic systems under the influence of lower concentrations of bile was evidently dependent chiefly on rapid physico-chemical changes on the surfaces, whereas the depression of their excitability under the action of higher concentrations was associated with slow biochemical processes and, in particular, with the blocking of protein SH groups by bile acids.

Taking account of recent findings indicating the importance of cholinergic systems in the activity of the nervous system [1, 5, 11], it may be postulated that the influence of low concentrations of bile and bile acids on the excitability of the nicotine-like cholinergic systems plays an important role in the mechanism of the neurotropic action of bile when retained in the body. This effect of bile must be borne in mind when bile preparations are used in medical practice.

SUMMARY

Bile in concentrations approximating those in the blood produced a marked effect on the nicotine-like cholinergic systems of the skeletal muscles. Higher concentrations of the bile acids (50-10 mg%) markedly decreased, and lower ones (0.1-0.005 mg%) distinctly increased the excitability of the muscles. The above effect of bile is associated with the bile acids which it contains. This effect, however, is stronger than that of cholic or of glycocholic acids. In this respect bilirubin is ineffective. The data obtained point to a possible mechanism of the neurotropic action of bile when retained in the organism, as well as when bile preparations are used in medical practice.

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