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Kinin forming and destroying activities in human bile and mucous membranes of the biliary tract

H. M. NIELSEN

Surgical Department L, Aarhus Municipal Hospital, University of Aarhus, Denmark

1. Human bile and tissue homogenates from the mucous membranes of the biliary tract possess plasma kinin forming activity (kininogenases) and plasma kinin destroying activity (kininases) in varying degrees.

2. The common bile duct, especially its lower part, had high kininase activity.

3. The liver possessed a high kininase activity, but no kinin forming activity.

4. The inactive precursor of plasma kinin, kininogen, was not detected in the bile.

5. Results from different pathological conditions are reported.

6. The implications of the findings are discussed. Special importance is attached to the question of a formation of kininogenases in the liver and to the significance of a plasma kinin activity in the bile and the biliary tract.

In spite of intensive research on the kallikrein-plasma kinin system, little has been published about its occurrence and importance in the bile and biliary tract. Amundsen & Nustad (1965) reported that rabbit bile possessed a pronounced kinin forming activity but that kininase activity was slight or non-existent. Apart from this observation there are no publications dealing with the occurrence of these activities in the walls of the biliary tract and no studies have been made with human material.

This paper describes results obtained with human bile and with homogenates of tissue from various parts of the human biliary tract.

Methods

Sixteen samples of bile from the gallbladder were studied. They were obtained by aspiration during or immediately after the surgical removal of the gallbladder. Five samples of bile from the common duct were obtained during operation, by aspiration before the injection of *X*-ray contrast media. Seventeen samples of bile were obtained from a drain in the common duct, from one to several days after the operation. Ten samples of mucous membrane were obtained from gallbladders removed surgically by gentle abrasion with a curette. Eighteen samples were taken from the wall of the common duct removed at autopsy. It was not possible to remove visible amounts of tissue with a curette, so the mucous membrane was cut away with a pair of fine scissors. Five samples of liver tissue were obtained at autopsy.

The tissue samples were washed several times in large volumes of saline. The samples of liver and common duct were homogenized in a Potter-Elvehjelm homogenizer. All tissue samples were then exposed to ultrasonic vibrations, 20,000 c/s for 4 min in an iced-water bath and centrifuged at 3,000 rev./min for 10 min. The sediment was discarded and the supernatant fluid diluted with saline until the protein concentration was 100 mg%. In most samples from the common duct, however, the protein concentration was less than 100 mg%.

Plasma kinin activity was measured on the isolated rat uterus preparation. The rats were treated with diethylstilboestrol (30 μg. intraperitoneally) 18–22 hr before being killed. The uterus was suspended in de Jalon solution containing methysergide (Deseril, Sandoz A.G.), $2{\cdot}13 \times 10^{-7}$M and mepyramine, $1{\cdot}25 \times 10^{-5}$M, in an organ bath of 10 ml. capacity. The temperature was 29° C. Doses were allowed to act for 45 sec and the interval between them was 5 min. The responses were compared with those caused by standard quantities of synthetic bradykinin (Sandoz A.G.).

Kinin forming activity was determined by incubating samples with a suitable substrate and assaying the kinin produced on the rat uterus. As described by Horton (1959), the fluid to be tested was first brought to pH 2·0 by the addition of 1 N HCl and kept at 37° C for 10 min. It was then converted back to its original pH by the addition of 1 N NaOH and an aliquot (0·4 ml.) incubated at 37° C with the substrate (0·6 ml.). Samples of the mixture (0·1 ml.) were removed every 5 min and tested for plasma kinin activity. The plasma preparation described by Amundsen, Nustad & Waaler (1963) was used as substrate and is referred to as " substrate 2 ". In addition, liver homogenates were tested with the substrate described by Jacobsen (1966), which reacts with plasma kallikrein. This is referred to as " substrate 1 ". In some experiments the samples and substrate were incubated in the presence of Aprotinin (Trasylol, Bayer A.G. ; 12·5 Kallikrein inhibitor units/ml. incubation mixture) and with soybean trypsin inhibitor (Fluka A.G. ; 100 μg/ml. incubation mixture) to see if these inhibited the formation of kinin.

Protein in the samples was estimated according to Lowry, Rosebrough, Farr & Randall (1951).

Kininase activity was determined by following the inactivation of synthetic bradykinin. The fluid to be tested (0·9 ml.) was incubated with 0·1 ml. of a solution of bradykinin (5 μg/ml. ; $4{\cdot}7 \times 10^{-6}$M) in saline at 37° C. Samples (0·1 ml.) were removed after 1, 6 and 11 min and tested for plasma kinin activity. Rugstad (1966, 1967) defines 1 kininase unit as the amount of kininase which inactivates 75% of the bradykinin in 11 min.

Results

Kinin forming activity was found in thirteen of the sixteen samples of bile taken from the gallbladder, in three of the five samples of bile from the common duct taken during surgery and in fifteen of the seventeen samples taken postoperatively. The degree of activity varied and the effects of a moderately active sample can be seen in Fig. 1.

The kininase activity of all the samples of bile varied from 1 to 8 u./ml. (Fig. 2).

Both kinin forming and kininase activity were present in the samples of mucous membrane from the gallbladder and the common duct. The kinin forming activity, however, was only slight and could not be detected in several cases. The kininase activity of the mucous membrane from the gallbladder varied between 1 and 6 u./mg protein wet weight. In the homogenates from the common duct the activity varied between 2 and 20 u./mg protein in the proximal part of the duct and was as high as 100 u./mg protein in the distal part, the average content of which was 40 u./mg protein.

The kininase activity of the five samples of liver homogenates studied varied between 3 and 6 u./mg protein. No kinin forming activity was detected.

The results are summarized in Table 1.

Kininogen was searched for in four bile samples, but could not be detected.

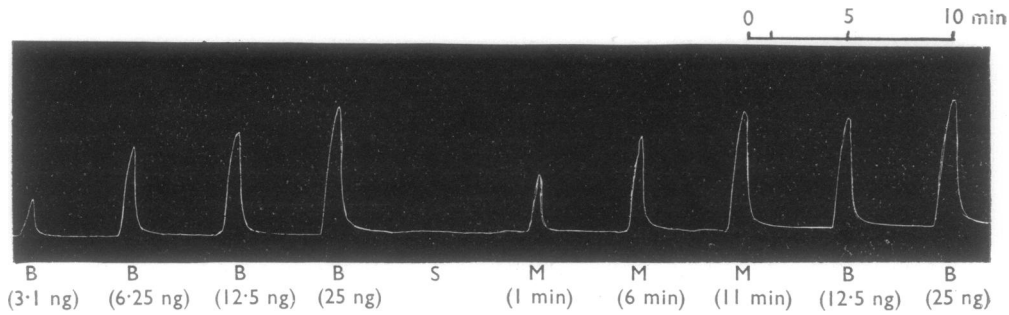

FIG. 1. Kinin forming activity of bile. 0·4 ml. of bile, after treatment with acid, was incubated at 37° C with 0·6 ml. of substrate plasma (substrate 2). The record shows the responses of the isolated rat uterus to doses of bradykinin (B) and to 0·1 ml. samples of the mixture (M), incubated for 1, 6 and 11 min. At S, 0·1 ml. of substrate plasma was tested.

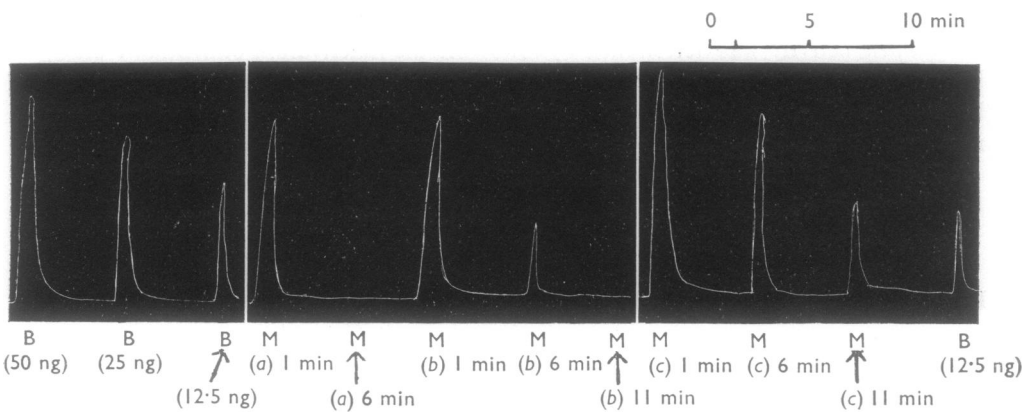

FIG. 2. Kininase activity in bile. 0·9 ml. of bile was incubated with 0·1 ml. bradykinin (5 μg/ml. in saline, $4{\cdot}7 \times 10^{-6}$M) at 37° C. 0·1 ml. samples of the incubation mixture (M) were tested for kinin activity after 1, 6 and 11 minutes. The record shows responses to bradykinin (B) and the inactivation of bradykinin in the incubation mixture by (*a*) undiluted bile, (*b*) bile diluted 1 : 3 with Tris buffer, pH 7·4 and (*c*) bile diluted 1 : 7.

The kinin forming activity of all the samples was similar in that it was greatly enhanced by pretreatment of the sample with acid (see **Methods**), was inhibited by Aprotinin and unaffected by soybean trypsin inhibitor (see **Methods**). The enzymes thus resemble glandular kallikrein.

The kininases present in the bile and in the homogenates of gallbladder, common duct and liver were all inhibited by $CoCl_2$ (3×10^{-3}M), $NiSO_4$ (3×10^{-4}M) and $ZnCl_2$ (3×10^{-4}M) and thus resemble cellular kininases (Amundsen & Nustad, 1964). Plasma kininases were unaffected by the concentrations of the inorganic salts.

In bile obtained from an encapsulated area around a perforated gallbladder no kininase activity was detected. In three patients with jaundice due to stones in the common duct, bile was aspirated from the duct during surgery. The bile was of a normal colour and had a high degree of kininase activity (12, 24 and 40 u./ml. respectively). On the other hand, " white bile " obtained from the common duct of several patients with obstruction of the duct by a carcinoma of the pancreas possessed neither kinin forming nor kininase activity.

Peritoneal fluid obtained from a patient with biliary peritonitis had no kinin forming activity and low kininase activity, less than 1 u./ml.

Discussion

Although the results do not enable conclusions to be drawn regarding the differences between normal and pathological conditions, they show that it is posible that kinin formation and kinin inactivation occur in the biliary tract, as they are known to do in mucous membrane cells in other parts of the alimentary canal (Amundsen & Nustad, 1965).

TABLE 1. *Kinin forming and destroying activities in human bile and homogenates of the biliary tract*

Liver	Kinin forming activity		Kininase activity
	No activity		3–6 u. (mean 4·6 u.)
	Number of samples	Bradykinin equivalent	
Gallbladder bile	2	>12·5,<25	
	4	12·5	1–6 u. (mean 3 u.)
	2	> 6·2,<12·5	
	5	> 3, < 6·2	
	3	No activity	
Common duct bile	2	25	1–8 u. (mean 4 u.)
	4	>12·5,<25	
	4	12·5	
	5	> 6·2,<12·5	
	3	> 3,< 6·2	
	4	No activity	
Gallbladder mucous membrane	3	6·2	
	3	> 3,< 6·2	1–6 u. (mean 2 u.)
	4	No activity	
Common duct mucous membrane	8 (0·2 mg protein/ml.)	No activity	Eighteen samples examined
	4 (0·5 mg protein/ml.)		upper part: 2–20 u. (mean 11 u.)
	1	> 3,< 6·2	
	2	3	lower part: 12–100 u. (mean 40 u.)
	1	No activity	

Kinin forming activity is expressed in "bradykinin equivalents", which indicate the amount of bradykinin, in ng, producing the same response on the rat uterus as 0·1 ml. of the mixture (0·4 ml. bile or homogenate +0·6 ml. substrate plasma) after incubation for 11 min at 37° C. Kininase activity is expressed in kininase units/ml. bile or /mg protein wet weight (for details see **Methods**).

The high kininase activity in the mucous membrane of the common duct is remarkable, but as all the samples were obtained at autopsies, it may arise from the pancreas. The tissue was washed several times, however, before ultrasonic treatment, and such a contamination is unlikely to account for the activity found in parts of the common duct far away from the pancreas.

The kinin forming activity of the mucous membrane of the common duct is difficult to assess because most of the samples had a low protein concentration (20 mg%). The results obtained with those samples which had a protein concentration of 50 mg%, however, suggest that it is unlikely to be high.

As the majority of bile samples from the common duct were obtained from a drain after surgery it is possible that their activity arises from a reflux of pancreatic juice, which has a high content of glandular kallikrein. In those cases where the bile was obtained by aspiration from the proximal part of the common duct during operation, however, kinin forming activity could be demonstrated in three out of five samples. It appears, therefore, that the bile itself is highly likely to be active, though it is not clear how this arises. It seems unlikely that the limited amount of enzyme present in the wall of the duct could produce much kinin forming activity in the bile during a rapid passage through the common duct. In the samples of " white bile ", produced by an obstructing carcinoma of the pancreas, there was no enzymatic activity.

Possibly the enzyme is formed in the liver, but so far no kininogenase activity has been proved to occur in this organ. Oates, Melmon, Sjoerdsma, Gillespie & Mason (1964) found a high kallikrein content in carcinoid metastases in the liver, but no kallikrein in normal liver tissue. Amundsen & Nustad (1965) in their investigations of rabbit and rat liver homogenates were not able to detect any kininogenase activity. It is generally assumed that plasma pre-kallikrein is formed in the liver, but there is no definite proof of this, nor could this explain the occurrence in the bile of glandular kallikrein activity, unless the activity could be due to a conversion of a plasma or liver precursor. The author has not been able to detect any kallikrein activity in the liver.

The physiological and patho-physiological significance of the described enzymatic activity is unknown, and the results presented in this paper give no clue to the possible roles of a plasma kinin activity in the biliary system. The bile contains kinin forming enzymes, but has no substrate (kininogen). On the other hand, plasma kinin formation must be possible in the walls of the biliary tract. Kinin activity here may influence blood flow, the state of tonus, evoke pain and so participate in the inflammatory mechanism. To clarify this, further investigations will be needed. These must include comparison of normal bile and normal mucous membranes with specimens from different pathological states, examination of the relationship between the enzymatic activity and the composition of the bile and experimental investigation of the influence of plasma kinins on the motility of the biliary tract.

I am indebted to Sandoz A.G. for the gift of synthetic bradykinin.

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