Plasma Villikinin.* (32071)

CHARLES L. JOHNSTON, JR., ESZTER KOKAS, AND E. S. BARROW

Division of Clinical Pathology, Medical College of Virginia, Richmond, and Department of Physiology, University of North Carolina School of Medicine, Chapel Hill

Recent studies have shown that potent villikinin preparations have been refined from canine and human intestinal mucosal extracts (1). A villikinin-like substance has been recovered from the urine(2-5). The electrophoretic mobilities of urinary and mucosal villikinin were shown to be identical(4). The villikinin-like substance excreted in the urine seems to be in the active form(4) and is designated urovillikinin.

Villikinin is produced in the small intestinal mucosa and effects its action on the villous motility. Crossed circulation experiments(6, 7) suggest that villikinin is transported in the general circulation. In some experiments with plasma fractions prepared by rivanol precipitation, charcoal adsorption, ultrafiltration, and continuous flow electrophoresis (8), it was found that one of the plasma fractions contained villikinin-like activity. Therefore, it seemed likely that villikinin might be recovered from the plasma by the technique used to extract it from the mucosa and from the urine.

Techniques similar to those used for the preparation of mucosal and urovillikinin were employed. These preparations were compared with mucosal and urovillikinin and their effects on villous motility assessed. Materials were obtained from humans as well as other species. It could be shown that the plasma contains a substance which, in the tests shown, has a villikinin-like activity similar to intestinal mucosal and urovillikinin.

Materials and methods. Preparation of crude villikinin. The acid extraction, charcoal adsorption technique described elsewhere(1) was used to prepare extracts from human, canine, and bovine intestinal mucosa; human and canine urine; and human, bovine and canine plasma. All were processed similarly regardless of the source and the extracts frozen at —20°C for lyophilized.

Refined extracts. The crude materials were desalted by electrolysis prior to fractionation by continuous flow electrophoresis as described previously(1). The resulting fractions, in barbital buffer, were stored at —20°C until thawed and injected into the test animal.

Villikinin assay. The villous motility in anesthetized fasting mongrel dogs was determined by observation, at 45× magnification, of the villous contractions in an exposed jejunal loop. The villikinin-like activity was evaluated by the changes produced in motility following intravenous injection of the material.

After establishing the basal motility, a control injection of a canine mucosal villikinin preparation of known potency was given. The effects of the test preparations were assessed by enumeration of the numbers of villous contractions per minute at stated post injection intervals. The changes from the basal motility were expressed in the contraction index, (C.I.).

$$\text{The C. I.} = \frac{\text{counts per minute—basal count}}{\text{basal count}}$$

Usually 5 or 6 injections could be evaluated in an individual experiment. At conclusion of the experiment, an injection of the control mucosal villikinin preparation was repeated to determine whether the reactivity of the animal had changed during the experiment. Details of this assay method are published elsewhere(1).

Results. Outdated human plasma was obtained from the blood bank and processed in 200 ml lots. The crude extracts were harvested by the processes used to obtain intestinal mucosal and urine extracts. Many such extracts were harvested and each was tested for its effect on villous motility. It was shown that all produced a pattern of reactivity similar to that shown for a crude mucosal extract.

* This study was supported in part by USPHS research grants AM-10427, AM-04675, and USPHS research career program award K3 GM 6786.

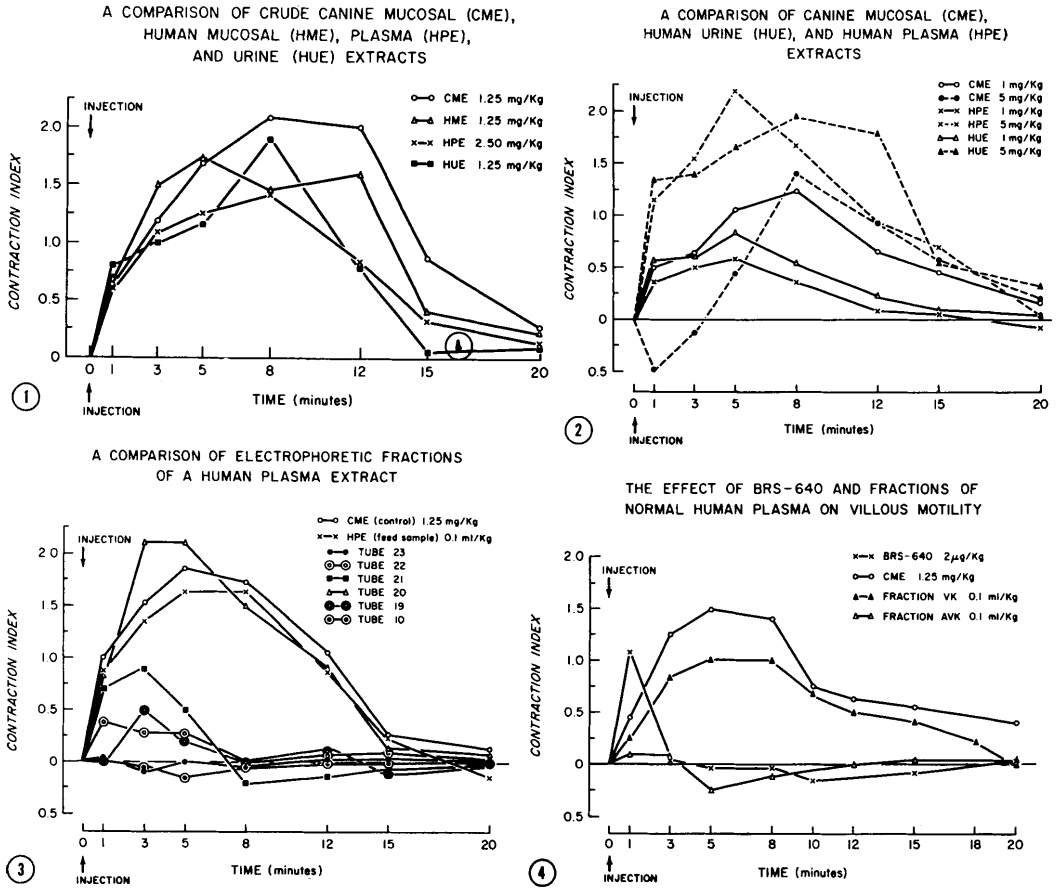

FIG. 1. Villous motility response following intravenous administration of crude villikinin preparations from different sources.

FIG. 2. Effect on villous motility of increased dosages of different crude villikinin preparations.

FIG. 3. Effect on villous motility of continous flow electrophoretic fractions compared with unfractionated plasma and mucosal villikinin preparations.

FIG. 4. Effect on villous motility of bradykinin compared with plasma and mucosal preparations.

However, it was noted that the amount required to produce what was considered to be a standard type response (Fig. 1) was variable and dose dependent. This suggested a difference of potency in individual extracts. Despite this, it established that plasma contained a material capable of markedly increasing villous motility. It should be noted that during this experiment no significant alterations of blood pressure or gross intestinal movements were noted.

In a previous communication(1) it was shown that canine and human intestinal villikinin preparations were behaviorally similar. The reports of villikinin in the urine (2-5) indicate that urovillikinin is very much like mucosal villikinin. The above experiments suggested that the material in plasma influencing villous motility appeared to be villikinin. In a series of experiments, one of which is shown in Fig. 1, the action of canine and human mucosal villikinin was compared with the action of extracts from human urine and plasma. Lyophilized preparations of each were reconstituted and injected in the amounts shown. It can be seen that the responses elicited following administration of each of the crude extracts were quite similar. It is not possible to tell from either the type or the magnitude of the response which extract was administered. It must be noted that the plasma preparation was given in twice the

concentration of the mucosal and urine extracts to achieve the standard type response.

In a recent experiment it was observed that crude mucosal extracts contain a substance inhibitory to intestinal villous motility(9). Its effect appears when an increased amount of a crude extract is given. To determine whether increased concentrations of plasma and urine extracts also might be inhibitory, preparations pooled from at least 10 individual fractionations were tested. Two different concentrations of canine mucosal, human urine and human plasma preparations were injected. The results are shown in Fig. 2. The canine mucosal extract in the two different concentrations used produced the changes observed previously in the villous motility. That is, 1 mg/Kg gave increased activity, whereas 5 mg/Kg gave an initial decrease which was followed by an increase and subsequent return to the basal level. Neither the plasma nor the urine preparation showed any evidence of such inhibition following administration in either the usual or the increased dosages. These results suggest that either the extracts do not contain the inhibitor or they have so little that it is ineffective in the production of inhibition at these concentrations.

Crude mucosal extracts can be fractionated by continuous flow electrophoresis to yield two fractions that influence villous motility (1,9). One of these, a rapidly moving fraction was identified as villikinin. The other a slowly migrating fraction inhibiting villikinin action was named antivillikinin(9). In further experiments, crude plasma extracts were fractionated in barbital buffer by using a one gram sample of a pooled preparation. The ninhydrin positive fractions were harvested and tested. Only one fraction was shown to influence villous motility. Under the conditions of this experiment, this was collected in tubes 19-23. The results are shown in Fig. 3. The maximal stimulating activity was found in tube No. 20. Tubes 19, 21, and 22 were less active. The maximal activity was comparable to that found in the unfractionated feed sample and in the mucosal villikinin extract control. One of the plasma fractions was shown to have "kinin-like", that is smooth muscle contracting, properties when tested on isolated guinea pig ileum. This was contained in tube No. 10. It can be seen that no significant alteration of villous motility followed its administration even though this corresponded to the fraction in mucosal extracts that contained the inhibitory material. None of the other fractions showed any evidence of inhibitor activity. Thus, it was found that the electrophoretic mobility of plasma villikinin is the same as that of mucosal villikinin. In similar experiments with crude urine extracts, identical results were obtained, namely villikinin in the rapidly moving fraction, and no inhibitory material demonstrable. Such data indicate that the different villikinin preparations evoke similar activity regardless of the source.

Substances other than villikinin produce changes in villous motility(10,11) but their basic actions are quite different from villikinin. Examples are the biogenic amines such as histamine and 5-hydroxytryptamine. In contrast to villikinin both substances influence blood pressure as well as peristaltic activity. Furthermore, these substances are adsorbed by charcoal and should be, and are, removed during the preparation of the crude fractions. Thus, they could be excluded as the villous active principle of the plasma.

It could be supposed that some "kinin-like" substances could be responsible for a villikinin-like activity. As noted above, one of the plasma fractions showed a "kinin-like" activity when tested on guinea pig ileum. Other results suggested that this fraction had a bradykinin-like activity. To determine whether this was bradykinin and what effect, if any, bradykinin had on villous motility, the following experimet was performed. Synthetic bradykinin (BRS-640, Sandoz) was compared with crude mucosal villikinin, and two refined electrophoretic fractions, VK and AVK. The results are shown in Fig. 4. The mucosal and VK preparations showed similar activities as would be expected since VK was a refined fraction similar to the one shown as tubes 19-23 in Fig. 3. AVK had "kinin-like" activity and was a fraction corresponding to tube 10, Fig. 3. This was without significant effect on villous motility. The bradykinin (BRS-640) produced an action on villous

motility quite different from that of any of the other preparations tested. The increase in motility was immediate, short-lived, and accompanied by a transient blood pressue depression of greater than 30 mm Hg. Blood pressure changes have not been seen following injection of villikinin(1). It can be concluded that bradykinin is not responsible for the villikinin-like action of plasma extracts.

The experiments shown were performed primarily using human materials. In addition to human plasma, extracts were made from bovine and canine plasma. Tests on these preparations similar to those outlined above gave identical results, as it was not possible to demonstrate any difference between human, bovine and canine plasma villikinin. Similarly it was not possible to demonstrate differences in human and canine urovilikinin. All of the villikinin preparations tested producted a reaction pattern which is similar to those shown in previous publications(1,9). While there may be differences in the amounts extracted from a particular source, *e.g.*, plasma, there can be little doubt that villikinin has been extracted from the plasma and that plasma villikinin is identical to mucosal and urovillikinin.

Summary. A series of experiments has been presented in which it was shown that crude extracts of human plasma contain a material that stimulates villous motility. It was not possible to distinguish a difference between the activities of crude mucosal, plasma, and urine preparations although it was suggested that there may be concentration differences. Refined preparations of villikinin showed a similarity to mucosal and urine extracts in activity as well as mobility. However, it was not possible to demonstrate antivillikinin in preparations other than those from intestinal mucosa. The activity of villikinin was different from that produced by histamine, 5-hydroxytryptamine and bradykinin. It is felt that the data presented clearly demonstrate the presence of villikinin in the plasma and that plasma villikinin is similar to mucosal and urovillikinin.

The authors wish to thank Mr. David Alford and Mr. Philip Arnold for their technical assistance in the performance of the experiments.

1. Kokas, E., Johnston, C. L., Jr., Am. J. Physiol., 1965, v208, 1196.
2. Svatos, A., Experientia, 1959, v15, 479.
3. Svatos, A., Queisnerová, M., Gáti, T., Arch. Int. Pharmacodyn. et Ther., 1960, v126, 315.
4. Svatos, A., Queisnerová, M., Vokac, V., Path. Biol., 1960, v8, 1193.
5. Ludány, G., Svatos, A., Gáti, T., Gelencsér, F., Arch. Int. Pharmacodyn., 1962, v140, 138.
6. Kokas, S., Ludány, G., Pfll. Arch. Geo. Physiol., 1933, v232, 293.
7. ———, Compt. Soc. Biol., 1934, v117, 972.
8. Kokas, E., Johnston, C. L., Jr., Barrow, E. S., Fed. Proc., 1963, v22, 225.
9. Kokas, E., Johnston, C. L., Jr., Arch. Int. Pharmacodyn., 1966, v160, 211.
10. Ludány, G., Kováts, J., Z. ges. exp. Med., 1940, v10, 287.
11. Ludány, G., Gáti, Szabó, St., Hideg, J., Arch. Int. Pharmacodyn., 1959, v118, 62.

Received January 27, 1967. P.S.E.B.M., 1967, v125.

Hepatic Microsomal Activities in Rats with Long and Short Sleeping Times after Hexobarbital: A Comparison.* (32072)

CHOZO MITOMA, SUSANNA E. NEUBAUER, NANCI L. BADGER, AND THEODORE J. SORICH, II (Introduced by J. H. Peters)
Biomedical Research Department, Stanford Research Institute, Menlo Park, Calif.

It is well known that the primary site of drug-metabolizing activities in the mammal is in the microsomal fraction of the liver. The microsome-catalyzed oxidative reactions such as hydroxylation of aromatic rings; oxidation of aliphatic side chains to alcohols, ketones, or carboxylic groups; dealkylation from oxy-

* This research was supported by Contract PH 43-65-1061 from Nat. Inst. of General Med. Sciences, USPHS.