Biochemical Pharmacology, 1965, Vol. 14, pp. 1161–1163. Pergamon Press Ltd., Printed in Great Britain.

SHORT COMMUNICATIONS

The estimation of the kallidins in blood and urine

(*Received* 14 *December* 1964; *accepted* 15 *March* 1965)

THE pharmacological properties of the kallidins and the location of specific kallikreins in most glandular tissue and in plasma suggest that local activation of this enzyme system may be involved in a variety of physiological and pathological conditions; i.e. blood flow regulation, inflammatory reactions, allergic phenomena, various kinds of shock, etc.[1] Direct determination of the polypeptide should provide a more sensitive means for the detection of activation of the kallikrein–kallidin system than measurement of the kallidinogen (bradykininogen) content of the plasma.[2, 3] This report describes a sensitive and specific method for the determination of the kallidins in blood and urine.

METHODS

Mongrel dogs anesthetized with sodium pentobarbital (30 mg/kg) were used as a source for blood and urine. Venous or arterial blood samples (usually 15 ml) were withdrawn within 20–40 sec into a syringe containing 1 M phosphoric acid (1·5 ml/10 ml blood) and soybean trypsin inhibitor (1·0 mg/10 ml blood). The blood (pH 4–5) was expelled immediately into a measuring cylinder, the volume recorded, and transferred with an equal volume of distilled water to a beaker containing 100 mg IRC-50 (H^+). The Amberlite IRC-50 was prepared from XE-64 by decantation; washing with acetone, sodium hydroxide, and hydrochloric acid;[4] and air-drying and screening through a 100-mesh sieve. The pH of the blood and Amberlite suspension was adjusted to 4·0 with 10 M formic acid and the mixture stirred for 1 hr at room temperature (22–26°). The resin which contained the adsorbed kallidin was recovered by filtration on a coarse, sintered glass funnel, washed with water, and transferred to a small beaker with the aid of 2–3 ml 0·2 M ammonium formate, pH 8·2. The pH of the mixture was adjusted with stirring to 8·5–9 with 10 M NH_4OH. The resin was removed by filtration through the sintered glass funnel, the eluate was adjusted to 8·2 with 10 M formic acid, the volume measured, and the sample frozen until assayed.

Urine was obtained by direct catheterization of the ureters of a dog through a mid-abdominal incision and 1–10 ml collected with stirring in a beaker containing 10 M formic acid (0·2 ml/10 ml urine). The volume of urine (pH 3–4) was recorded and transferred with 30–40 ml distilled water to a beaker containing 100 mg IRC-50 (H^+). The conditions for adsorption and elution are identical with those described for blood.

Bioassay of the polypeptide was conducted on the isolated rat uterus. Female rats weighing 180–250 g were injected with stilbesterol (10 μg/100 g) 18 hr before use. A uterine horn was suspended in a 10-ml bath[5] containing de Jalon's solution[6] at 22–29°. The test solutions were initially assayed for their ability to contract the rat uterus as compared to kallidin-9 (bradykinin, Sandoz Laboratories) and then identified as a kallidin by bioassay after incubation of the test solution with heparinized guinea pig plasma at a ratio of 0·01 ml plasma to 10 ng oxytocic substances calculated as kallidin-9. The heparinized guinea pig blood was obtained from animals lightly anesthetized with ether. The plasma was separated immediately, adjusted to pH 7·0 with 1·0 M HCl, and stored at −10° when not in use.

RESULTS AND DISCUSSION

Several oxytocic substances, which might be released endogenously and interfere with the determination of kallidin in biological fluids, were examined for their ability to be adsorbed and eluted from IRC-50. Neither acetylcholine nor serotonin could be detected in the eluates (Table 1). However, angiotensin II and angiotensin II amide, like the kallidins, were readily adsorbed and eluted

TABLE 1. RECOVERY OF OXYTOCIC SUBSTANCES FROM IRC–50 AND THEIR INACTIVATION WITH PROTEOLYTIC ENZYMES

Oxytocic substance*	Concentration (μg)	Recovery (%)	% Inhibition of activity with		
			Chymotrypsin†	Trypsin†	Guinea pig plasma
Acetylcholine	200, 1000	<1			
Serotonin	20, 50	<2			
Angiotensin II	0·5–1	65–95	>80	62–80	0
Angiotensin II amide	0·5–2·5	80–95	>80	>80	0
Kallidin–11	0·6–1·5	65–85	>80	0	>80
Kallidin–10	0·2–0·4	55–72	>80	0	>80
Kallidin–9	0·1–0·25	65–85	>80	0	>80

* Acetylcholine chloride (Merck & Co., Inc.); creatinine sulfate complex of serotonin (5-hydroxytryptamine, Calif. Corp. Biochem. Res.); α-1-aspartyl[1]-valyl[5]-angiotensin II and α-1-asparaginyl[1]-valyl[5]-angiotensin II amide (hypertensin) (CIBA, Ltd., Basle, Switzerland); kallidin-11 (methionyl kallidin-10, Dr. E. Schroder, Schering A.G. West Berlin, Germany); kallidin-10 and -9 (Dr. E. D. Nicolaides, Parke, Davis & Co., Ann Arbor, Mich.). In order to ensure stability of the compounds, the angiotensins and the kallidins were dissolved and diluted in sterile 0·25% solution of casein (Hammersten).

† The polypeptides (5–50 ng) were incubated at 22–24° with 50 μg chymotrypsin (Nutritional Biochemicals Corp. salt-free from ethanol) for 4 min or with 100 μg trypsin (Worthington Biochemical Corp., 2 times crystalline, salt-free for 1 min. Excess trypsin was inhibited by the addition of 1·0 mg ovomucoid trypsin inhibitor (Mann Res. Lab. Inc.) for 1 min. Samples were compared to the appropriate controls, substituting water for enzyme.

from this resin. Since these polypeptides are nearly as potent as the kallidins in their ability to contract the rat uterus, methods to distinguish the two classes of polypeptides were sought. Brief incubation with chymotrypsin readily inactivates both types of polypeptides, indicating that this enzyme cannot be used for specificity determinations of the kallidins.[7, 8] Trypsin, on the other hand, specifically destroys the angiotensins, under the described conditions (Table 1). However, with more prolonged incubation, trypsin preparations were capable of inactivating the kallidins, presumably owing to contamination with small amounts of chymotrypsin or other pancreatic proteinases.

Specific destruction of the kallidins was obtained by incubating them with guinea pig plasma. This plasma is the most potent source of kallidinase (carboxypeptidase N),[9, 10] and similar results can probably be obtained by using DFP-treated carboxypeptidase B (Worthington Biochemical Corp.). Guinea pig plasma also contains enzymes which will inactivate the angiotensins.[11] In agreement with other investigators,[12] it was observed that angiotensin II was destroyed at a different rate than was angiotensin II amide, suggesting that different enzymes were involved in the destruction of the two octapeptides and that biological investigation of angiotensin inactivation should be conducted, employing only the naturally occurring pressor polypeptide.[13] The rate of inactivation of the angiotensins, however, was relatively slow when compared to that of the kallidins, so that a concentration of guinea pig plasma could easily be chosen to differentiate between the two types of polypeptides (Table 1).

The method for the determination of the kallidins (which has been described in detail under Methods) is based initially on an attempt to prevent both destruction of the kallidins and activation of plasma kallikrein, by rapid adjustment of the pH and by the presence of soybean trypsin inhibitor. The kallidins are then partially purified and concentrated by adsorption on IRC-50. This resin has been shown to be an effective agent for the adsorption of the kallidins at pH 4–6[14] and has been used successfully by a number of authors[7, 15, 16] for both batch and column adsorption of these peptides. The recovery of added kallidin from arterial and venous blood and from urine was determined in a number of experiments, as illustrated in Table 2. The recoveries were in general lower in the presence of blood than in its absence (compare Tables 1 and 2). In these experiments kallidin was added to the syringe prior to the addition of the blood so that the lower rate of recovery may reflect some destruction of the polypeptide during the brief interval in which the sample was obtained. Also, at levels of 20–75 ng/ml the recovery of polypeptide varied in different dogs from 24–53%, whereas repeated determinations in the same animal varied less. Similar differences have been reported in humans.[17]

TABLE 2. RECOVERY OF KALLIDIN FROM CANINE BLOOD AND URINE

Source	Kallidin added (ng/ml)	Recovery* (%)
Blood	75	35; 34,46
	20	53,47; 24,28; 30,35
	8	28; 46; 14
	2	20,30
Urine	1,000	56; 50

* Numbers separated by commas represent duplicate determinations in one animal.

At levels of kallidin above 8 ng/ml no interference was noted from other oxytocic substances in blood. However, below these levels guinea pig plasma failed to inactivate all the oxytocic acitivity, making quantitation of the samples more difficult. In agreement with other investigators,[8, 18] normal canine blood contains no or, at best, traces of kallidin. This contrasts sharply with the high levels reported in the human.[7, 17] However, in this laboratory high concentrations of kallidin have not as yet been encountered in human blood. In blood from three patients with the carcinoid syndrome, the kallidin concentration was less than 2·5 ng/ml, and recovery of added polypeptide was within the expected range (27–40%). If these low values are substantiated by a larger series of individuals, it would appear that the higher levels encountered in earlier studies may have been due to activation of plasma kallikrein during withdrawal of the blood.

Acknowledgement—The technical assistance of W. Anderson, Jr., and W. D. Fisher is gratefully acknowledged.

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