the inhibition of inosine permeation and a consequent decrease in the availability of inosine as a substrate of conversion to hypoxanthine.

The present study leads to the conclusion that, in the functioning heart, as in isolated cells, the primary effect of Persantin at a low concentration is on the permeation of nucleosides rather than on the metabolism of nucleosides in the cell.

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Biochemical Pharmacology, Vol. 22, pp. 1121–1123. Pergamon Press, 1973. Printed in Great Britain.

Subcellular location of the kininogenase in the coagulating gland of the guinea-pig*

(*Received* 18 *August* 1972; *accepted* 3 *November* 1972)

THE COAGULATING glands of the guinea-pig are paired accessory sex glands situated near the base of the seminal vesicles.[1] The secretion from these glands was first shown to be highly "toxic" to laboratory animals by Freund *et al.*[2,3] They also observed that it increased vascular permeability, possessed arginine esterase activity, and coagulated the secretion of the seminal vesicles. In further studies, it was shown that the substances responsible for these effects were proteins and that the coagulating action was due to a different protein from that responsible for the other effects of the secretion.[3,4]

Bhoola *et al.*[5] and Moriwaki and Schachter[6] later found that the accessory sex glands of the guinea-pig, and the coagulating gland in particular, contain a potent kininogenase. They showed that this kininogenase, called CGK, was reponsible for the hypotensive, permeability-enhancing, and esterolytic actions of extracts of the coagulating gland of the guinea-pig. They also showed that CGK released the nonapeptide, bradykinin.

The main purpose of the present experiments was to determine the subcellular location of this kininogenase. Recent studies of this nature have been done for the kininogenases of the submaxillary gland,[7–9] pancreas[10] and kidney.[11,12] In the submaxillary gland and pancreas of several mammals studied, the enzyme was found mainly in zymogen-type granules.[7–10] In the kidney of the rat, it has

* This work was supported by a grant from the Medical Research Council of Canada.

separately been reported to be present mainly in the lysosomal[11] and microsomal fractions[12] respectively. In the present study on the coagulating gland of the guinea-pig, however, practically all (91–99 per cent) of the kininogenase recovered was in the soluble, nonparticulate fraction.

The following procedures and methods were employed. Adult male guinea-pigs (450–1000 g) were killed by a blow on the head and exsanguinated by section of the jugular vein. The coagulating glands (150–200 mg from each animal) were then removed and homogenized in 0·25 M sucrose (20–30 mg ml^{-1}) using a Teflon–glass homogenizer. Approximately one-tenth of the homogenate was kept for reference and the remainder was separated into a particulate fraction, F_p, and a supernatant (or soluble) fraction, F_s. Separation was effected in a Beckman L3-50 centrifuge, with a type 50 rotor at 147,000 *g* for 60 min at 4°. The supernatant solutions and particulate deposits were freeze-dried and kept at 4° for subsequent measurements of enzyme distribution in the two fractions.

Kininogenase activity of the particulate (F_p) and soluble (F_s) fractions was measured by both biological and chemical methods. Biological assay was performed using the isolated guinea-pig ileum preparation to measure the kinin released from dog plasma globulin as substrate;[13] the kininase, also present in the homogenates and which would interfere with assay of the kinin released,[5] was inactivated by acidification of the fraction to pH 2 for 30 min, after which it was neutralized to pH 7.[14] Chemical measurement of kininogenase activity was made using the enzymatic hydrolysis of *a*-*N*-benzoyl-L-arginine ethyl ester, BAEe.[15] Kininase was estimated[6] by incubating several concentrations of each fraction with bradykinin under standard conditions and then determining the amount of each fraction which produced a 50 per cent reduction of the added bradykinin. The percentage distributions of kininase in F_p and F_s, respectively, were calculated from this measurement. Other enzymes measured were acid phosphatase[16] and succinic dehydrogenase.[17] Approximately 500 mg of fresh tissue was used for each experiment shown in Table 1.

TABLE 1. ENZYME ACTIVITIES IN PARTICULATE AND SOLUBLE FRACTIONS FROM HOMOGENATES OF GUINEA-PIG COAGULATING GLAND

Experiment No.	Kininogenase (CGK) Bioassay (% total recovery)		Kininogenase (CGK) Chemical assay (% total recovery)		Kininase (% total recovery)		Acid phosphatase (% total recovery)	
	F_p*	F_s*	F_p	F_s	F_p	F_s	F_p	F_s
1	0·5	99·5						
2	2	98						
3	2	98						
4	9	91						
5			1	99	63	37		
6			2	98	90	10		
7			6	94	81	19		
8			4	96	70	30		
9			2	98			79	21
10			1	99	91	9	59	41

* F_p = particulate fraction; F_s = supernatant fraction.

In all ten experiments, the kininogenase activity was recovered mainly in the supernatant solution after centrifugation of the homogenates at 147,000 *g* for 60 min. Bioassay and chemical measurements of kininogenase did not differ significantly. The mean distribution of kininogenase was 97 per cent in the supernatant solutions and 3 per cent in the particulate deposits. These results are illustrated in Table 1. The particulate deposit was not separated further in view of its extremely low content of kininogenase. The mean recovery of the total kininogenase activity of the original homogenates was 113 per cent (range, 82–176 per cent in ten experiments).

Since previous workers[7–12] found that the kininogenases of submaxillary gland, pancreas and kidney were located mainly in subcellular particles, albeit different ones, our results were somewhat surprising. The possiblity that unusual breakdown of particles was occurring in the case of the coagulating gland was, therefore, carefully considered. Of relevance in this connection is the observation that membranes of zymogen granules from the rat parotid gland become "leaky" at low temperature;[18]

also, the kallikrein-containing granules from the submaxillary gland of the rat[7] and mouse[19] are more stable at room temperature than at 4°. In one experiment, therefore, the procedures were carried out at 20° instead of at 4°; in this experiment (No. 3, Table 1), 98 per cent of the CGK recovered was in the supernatant solution. In another experiment (No. 4, Table 1), the glands were homogenized in 0·7 M instead of 0·25 M sucrose in a further attempt to reduce possible rupture of particles; 91 per cent of the CGK was still found in the supernatant solution.

Further evidence that our procedures preserved the relative intactness of particles is evident from our measurements of kininase. In the case of kininase, unlike CGK, the activity was largely in the particulate deposits, varying from 63 to 91 per cent in five separate experiments. Similarly, in two experiments, acid phosphatase, an enzyme known to be associated with lysosomal particles, was recovered in the particulate fraction to the extent of 59 and 79 per cent, respectively. These results are shown in Table 1. Also, in another single experiment, 66 per cent of the succinic dehydrogenase, a mitochondrial enzyme, was recovered in F_p.

Our results are in agreement with and extend those of Gotterer *et al.*[4] They found that extracts of the accessory sex glands of the rat and guinea-pig hydrolyzed synthetic arginine esters (TAMe) at very rapid rates; most of this activity in the case of the guinea-pig's coagulating gland was also recovered in the supernatant solution after centrifugation at 3500 *g* for 20 min at 20°. They obtained similar results for the ventral prostate gland of the rat.[4]

The results of the present study do not indicate the physiological role of CGK. This enzyme, however, cannot have a common function in the sex glands of different mammals, at least not one related to its ability to release a kinin, since only in the guinea-pig does the arginine esterase in the sex glands possess the additional property of releasing a kinin.[5,6] What is now clear, however, is that the kininogenases from different sources differ not only in their molecular weights, amino acid compositions and substrate specificities,[20] but also in their subcellular locations. The possibility must be considered, therefore, that they also have different physiological roles. Indeed, like that of the kininogenase, trypsin, their physiological significance may even be unrelated in some instances to their ability to release a kinin.

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