

DIFFERENTIAL INACTIVATION OF ADENOSINE BY HUMAN AND CANINE BLOOD

H.VAN BELLE

*Department of Biochemistry, Janssen Pharmaceutica,
Beerse, Belgium*

Received 26 March 1969

1. Introduction

According to Berne's hypothesis on the metabolic regulation of coronary blood flow, adenosine is the main vasoactive substance released from the myocardial cell during hypoxia [1]. The dilatory effect of exogenous adenosine on the coronary vessels is short-lived, due to its rapid inactivation in the blood and/or tissues. Using a new and fully automatic colorimetric method for the continuous determination of adenosine [2], we have found marked qualitative and quantitative differences in the rate of disappearance of adenosine from human and canine blood. The present paper reports on these differences, and shows that although in human blood adenosine is mainly taken up by the erythrocytes, in canine blood it is mainly taken up by the platelets.

2. Materials and methods

Approximately 8 ml of blood from healthy male human volunteers or from male beagle dogs was collected by free flow from a venipuncture into a plastic tube containing 2 ml of ACD (acid-citrate-dextrose: USP XVI, 662). All manipulations were performed using plastic syringes or tubes, and care was taken to avoid haemolysis.

2.1. Preparation of total blood samples

0.1 ml of human blood or 2 ml of canine blood was diluted to 8 ml with isotonic phosphate buffer (pH 7.4).

2.2. Preparation of erythrocyte samples

Total blood samples were centrifuged for 15 min at 350 g to separate the erythrocytes and the platelet-rich plasma. The sedimented red cells were then diluted with physiological saline at pH 7.4, and approximately 8 ml was layered onto 25% Ficoll (Pharmacia, Uppsala) in saline. The erythrocytes were separated from the residual platelets either by allowing the mixture to stand for several hours or by centrifuging at low speed. The erythrocytes thus obtained were washed twice with saline, taken up in 3 ml of saline, and aliquots of 0.1 ml (human) or 1 ml (canine) of the suspension were diluted to 8 ml with isotonic phosphate buffer.

2.3. Preparation of plasma samples

The platelet-rich plasma was centrifuged for 25 min at 750 g to sediment most of the platelets, and a further 20 min at 2500 g to remove the remainder: 2 ml of the supernatant was then added to 6 ml of phosphate buffer.

2.4. Preparation of platelet samples

The platelet sediment was washed 3 times with physiological saline by sucking up and down in a syringe and centrifuging. Aliquots of 1 ml (human) or 0.5 ml (canine) of a suspension of the residue in 3 ml of saline were diluted to 8 ml.

2.5. Preparation of lysates of total blood, erythrocytes and platelets

Portions of the total blood, washed erythrocytes or platelets were diluted with water (1:4, v/v) and

Table 2
Rate of inactivation of adenosine by human and canine blood and blood fractions at pH 7.4 and 37°C.

Fraction	Rate of adenosine inactivation by stated fraction (μmoles/hr)				Ratio of lysed/intact blood	
	Intact blood		Lysed blood			
	Human	Canine	Human	Canine	Human	Canine
1 ml of total blood	10.5 ± 0.9	1.04 ± 0.16	11.4 ± 0.8	18.4 ± 1.6	1.09	17.7
10 ⁹ erythrocytes	2.23	0.07	2.34	0.40	1.05	5.71
Erythrocytes/ml of total blood*	9.81	0.43	10.30	2.44		
10 ⁸ platelets	0	0.39	0	4.87		
Platelets/ml of total blood*	0	1.29	0	16.07	—	12.5
1 ml of plasma	0.20	0.29	—	—	—	—
Plasma/ml of total blood	0.11	0.15	—	—		

* Calculated from the results given in table 1.

Table 3
Relative potencies of the various blood fractions as adenosine inactivators (total blood = 100).

Fraction	Intact blood		Lysed blood	
	Human	Canine	Human	Canine
Total blood	100	100	100	100
Erythrocytes	93.5	41.3	90.4	13.3
Platelets	0	124	0	87.3
Plasma	1.0	14.4	1.0	0.8

Calculated from the results given in table 2.

Haemolysis did not appreciably alter the rate of adenosine inactivation by total human blood (table 2). This finding is in agreement with those of Bunag et al. [3], but contrary to those of Koss et al. [4] who observed a 75% increase in activity on lysis. Haemolysis of canine blood caused a considerable increase in the rate of adenosine inactivation: approximately 18-fold in the present experiments, as compared with 8-fold in those of Koss et al. [4] and 10-fold in those of Kübler and Bretschneider [5]. The lower values reported for the earlier studies may have been due in part to incomplete lysis of the platelets (see below).

4.2. Plasma

The absolute level of adenosine deaminase activity in plasma was low for both species (table 2); as a per-

centage of the total (intact) blood activity it was about 1% in humans and about 14% in dogs (table 3). These results are in broad agreement with those of Koss et al. [5].

4.3. Erythrocytes and platelets

It is generally assumed that the erythrocytes are largely responsible for the uptake of adenosine from both human and canine blood [3–7]. However, table 3 shows that in the present study canine erythrocytes accounted for only a small part of the adenosine inactivation potency of the blood, and even this small part may be questioned in view of the difficulty of ridding the erythrocytes completely of platelets. Indeed, in a subsidiary experiment using canine erythrocytes sedimented 3 times in Ficoll, we did not observe any adenosine uptake by the cells or any adenosine deaminase activity on lysis.

The platelet fractions of canine blood, intact or lysed, showed much the greatest activity (table 3). The higher potency of the intact platelet fraction than of the total blood sample may have been due to adsorption from solution of some of the adenosine in the latter, perhaps onto the erythrocytes. By contrast, for human blood, the platelet fraction had negligible activity, and over 90% of the total potency resided in the erythrocyte fraction.

Further studies are in progress to investigate the differences in activity of the various blood fractions of other species.

Acknowledgements

The author is indebted to Dr. P.A.J.Janssen, Director of the Laboratory, for his continuous encouragement, and to Dr. A.E.F.Chandler for his help in the preparation of the manuscript.

References

- [1] R.M.Berne, Am. J. Physiol. 204 (1963) 317.
- [2] H. Van Belle, in manuscript.
- [3] R.D.Bunag, C.R.Douglas, S.Imai and R.M.Berne, Circulation Res. 15 (1964) 83.
- [4] F.W.Koss, G.Beisenherz and R.Maerkisch, Arzneimittel-Forsch. 12 (1962) 1130.
- [5] W.Kübler and H.J.Bretschneider, Pflugers Arch. Ges. Physiol. 277 (1963) 141.
- [6] W.Kübler and H.J.Bretschneider, Pflugers Arch. Ges. Physiol. 280 (1964) 141.
- [7] H.Stormann, Arzneimittel-Forsch. 16 (1966) 705.