JOURNAL OF PHYSIOLOGY AND PHARMACOLOGY 1993, 44, 2, 171—177

J. ŚWIĘS, S. CHŁOPICKI, R.J. GRYGLEWSKI

KININS AND THROMBOLYSIS

Department of Pharmacology, University School of Medicine, Kraków, Poland

In cats with extracorporeal circulation arterial blood pressure and thrombolysis were assayed. In this model apart from their hypotensive properties kallikrein (3-10 units/kg, i.v.) and captopril (> 200 μg/kg, i.v.) dissipated blood clots which were preformed on superfused collagen strips. Captopril at a lower dose of 50 μg/kg i.v. potentiated the thrombolytic effect of kallikrein while aprotynin (100.000 unit/kg, i.v.) abolished it.

Thrombolysis by kallikrein was mediated by an unstable principle which was decomposed by blood during 15 min of incubation at 37°C. Generation of this principle was inhibited by pretreatment of animals with aspirin (50 mg/kg, i.v.). The above analysis points to prostacyclin which owing to its platelet-suppressant and fibrinolytic properties induces thrombolysis when released by kinins.

Key words: *kinins, kallikrein, captopril, aprotynin, aspirin, prostacyclin, clots, thrombolysis, platelets.*

INTRODUCTION

Kallikrein (Padutin) has been used for treatment of critical ischaemia in peripheral vascular disease (1, 2). An apparent exaplanation of its therapeutical efficacy was an assumption of increased blood flow through the ischaemic area owing to the generation of kinins (3) which in turn might elicit vasodilation through the endothelial release of prostacyclin and nitric oxide (4). Captopril, an inhibitor of angiotensin converting enzyme (ACE) (5) is a vasodilator because ACE apart from its name-defined property is also known to inactivate kinins (6). We have published experimental (7) and clinical (8) data pointing to platelet-suppressant, fibrinolytic and thrombolytic potentials of kallikrein and captopril. Presently, an evidence for a possible mechanism of thrombolysis by kinins is presented and it is proposed that the therapy with kallikrein or captopril may be beneficial not only because of vasodilation but also because of their thrombolytic effects.

MATERIALS AND METHODS

Thirty one cats of either sex were anaesthetized with sodium pentobarbital (30 mg/kg, i.p.) and heparinized (2500 U/kg i.v.). Mean arterial blood pressure was recorded from the right carotid artery by a Statham transducer. Blood thrombi were formed on collagen strips superfused with arterial blood from the right carotid artery (3 ml/min). Blood after superfusion was returned to the venous system of the animal. The weight of thrombi (300-600 mg) was continuously monitored by a modified Harvard transducer of the type 386 (9).

Alternatively, two collagen strips were superfused with two arterial blood streams. The first one reached its collagen strip along the shortest pathway from the left carotid artery i.e after 60 sec of flowing in a tubing (DIRECT). The second blood stream was allowed to circulate in a warmed (37°C) silicone coil for 15 min (DELAY) before blood would reach its strip. Drugs were injected either into femoral vein (i.v.) or, locally, into blood that superfused collagen strips (O.T.).

In our *in vivo* system prostacyclin (0.3-0.5 μg/kg, i.v.) had a dose dependent thrombolytic ation which appeared as a transient (10-30 min) loss in weight of a DIRECT but not of a DELAY strip, although both detectors were sensitive to the thrombolytic action of locally administered prostacyclin (10-30 ng O.T) (*Fig. 1*). This labile thrombolytic action is unique not only for

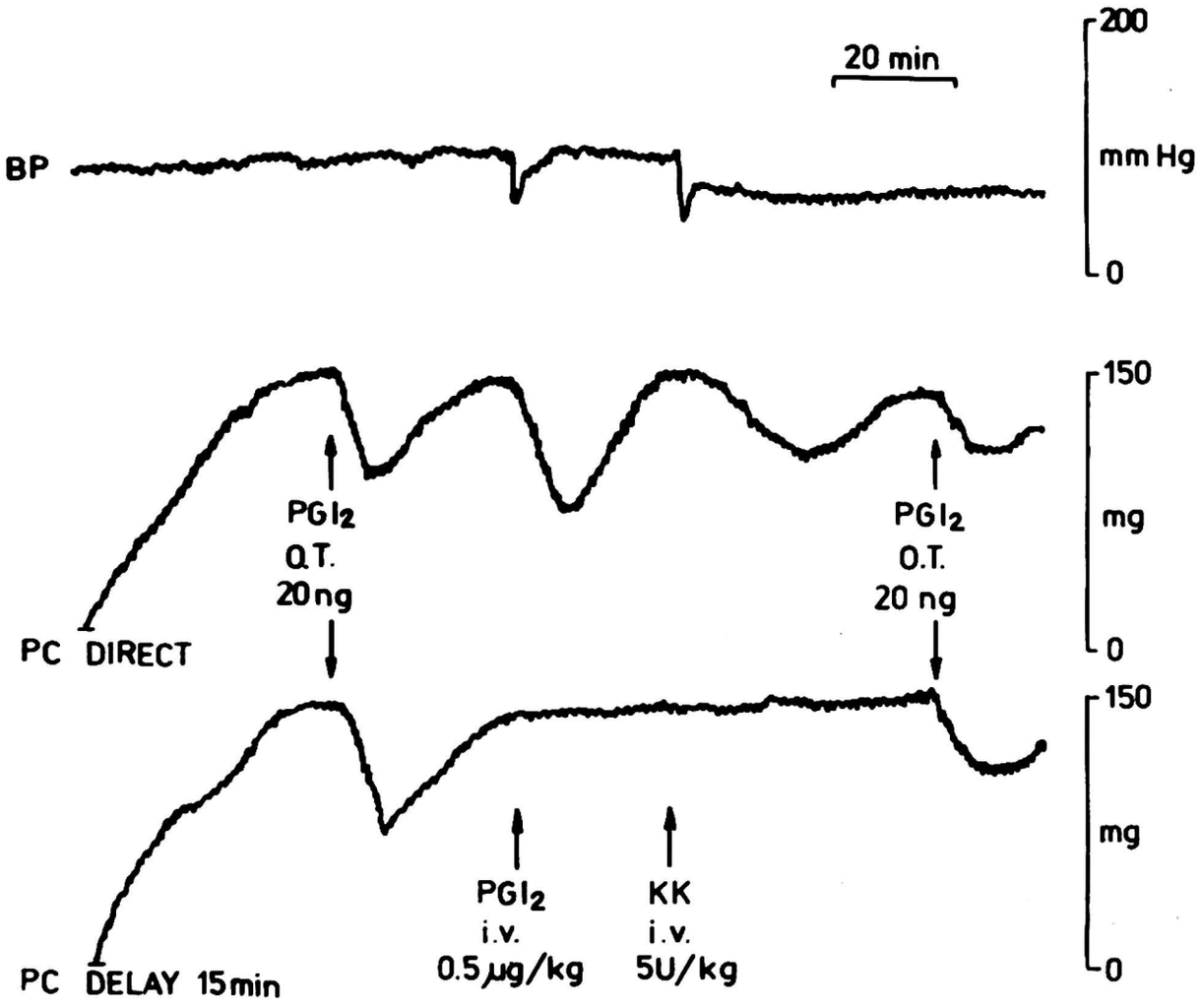

Fig. 1. The thrombolytic action of prostacyclin (PGI_2) given O.T. and i.v. and kallikrein (KK) given i.v. Two detectors of thrombolytic action (PC DIRECT and PC DELAY) were used. The first one was direcly superfused with arterial blood while the second strip was superfused with blood which had been circulated for 15 min 37°C in a delay coil. The sensitivity of both detectors was checked with prostacyclin (PGI_2) O.T at the beginning and at the end of the experiment. In between prostacyclin (PGI_2) and kallikrein (KK) were injected intravenously. Note a similarity of responses to PGI_2 and kallikrein (KK) on BP, PC DIRECT and PC DELAY.

exogenus prostacyclin administered systemically (10) but also for endogenous prostacyclin that may have been released into circulation and characterized as follows (11, 12). Firstly, its release is blocked by aspirin (ASA, 50 mg/kg, i.v.) given 5-10 min before the administration of a releaser such as methacholine, bradykinin or nicotinamide. Secondly, at its active thrombolytic concentration a PGI_2 releaser does not induce thrombolysis when given O.T. We had satisfied the requirements of the bioassay of endogenous prostacyclin (nicotinamide 50-100 mg/kg, i.v.) (12) before we tried kallikrein and captopril as potential releasers of endogenous prostacyclin.
Statistical analysis. Results are expressed as means $\pm$ S.D. of n observations. Statistical difference between means were assessed using unpaired two-way Student's test. A p value less than 0.05 was considered statistically significant.

RESULTS

Kallikrein (3-10 U/kg, i.v.) had a transient thrombolytic action comparable to that of prostacyclin (0.5-1.0 μg/kg, i.v. or 10-20 ng O.T.) and produced a long-lasting decrease in mean arterial blood pressure (*Fig. 2*). The thrombolytic effect of kallikrein was enhanced by pretreatment with a low dose of captopril (50 μg/kg, i.v.). Kallikrein at a concentration of 0.1 U/ml infused O.T. produced neither thrombolysis nor hypotension. Like exogenous and endogenous (released by nicotinamide) prostacyclin, a thrombolytic principle released by kallikrein did not survive 15 min of the incubation in blood at 37°C. The thrombolytic action of kallikrein (5-10 U/kg, i.v.) was abolished by pretreatment of the animal with aprotynin (100.000 U/kg, i.v.) or with ASA (50 mg/kg, i.v.). The hypotensive action of kallikrein was also abolished by

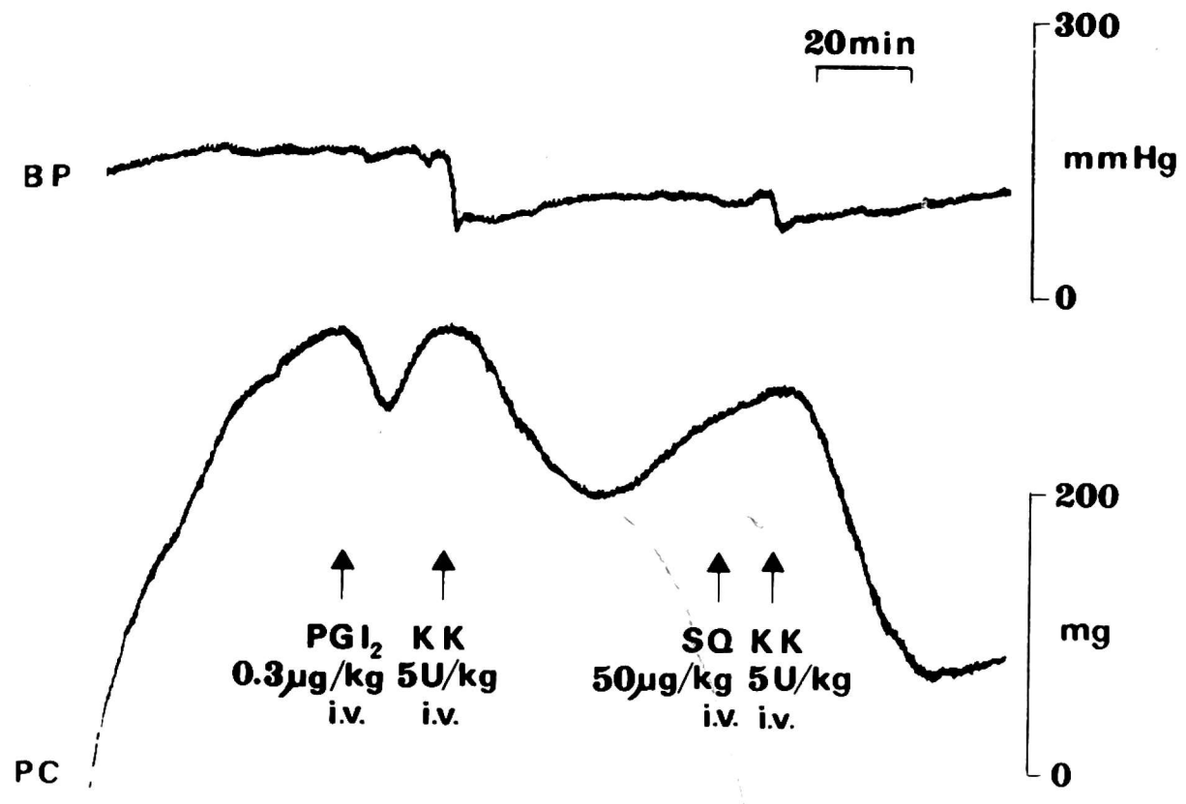

Fig. 2. The potentiation of the thrombolytic action of kallikrein (KK) by a low dose of captopril (SQ). For abbreviations see *Fig. 1.*

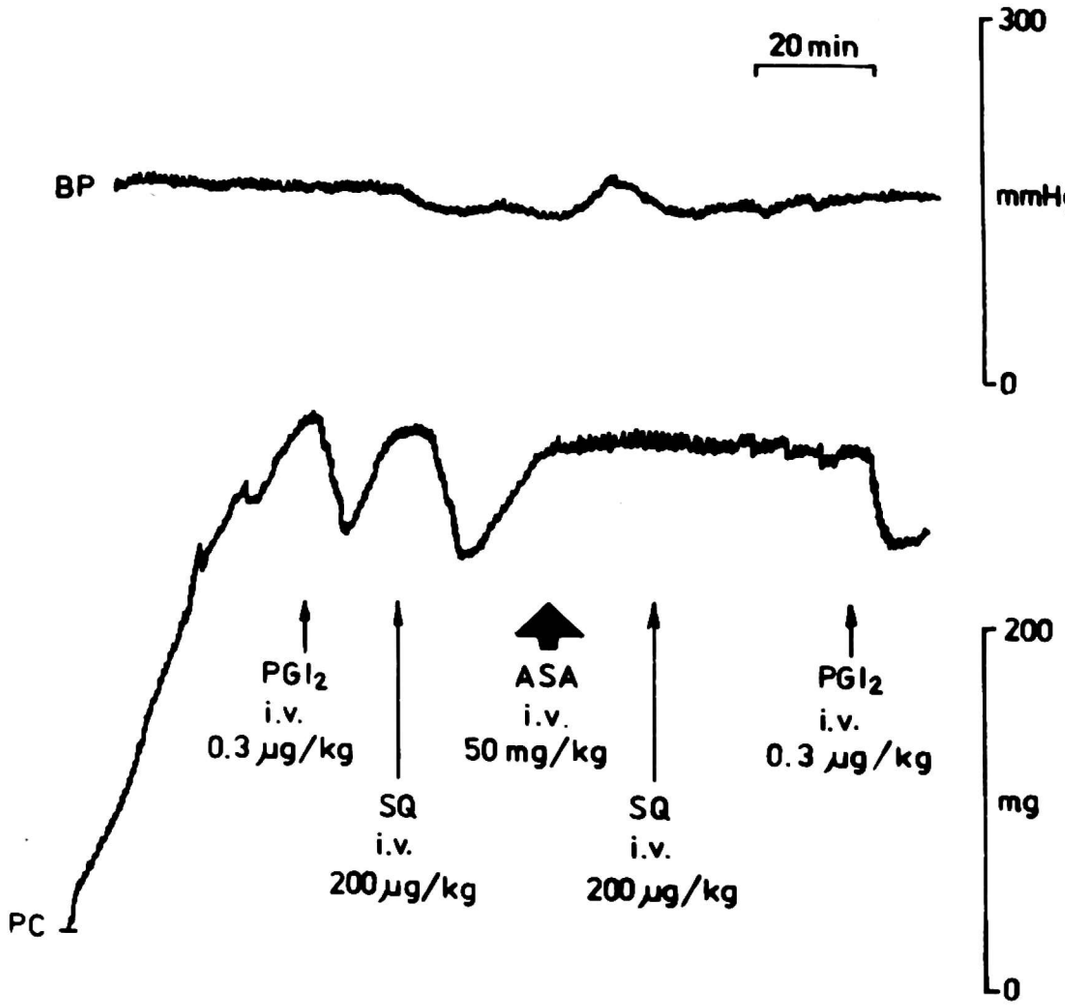

Fig. 3. The inhibition of the thrombolytic action of a high dose of captopril (SQ) by acetylsalicylic acid (ASA). Note that ASA blocked the thrombolytic effect of SQ but not that of exogenous prostacyclin (PGI_2).

Table 1. The effect of kallikrein, captopril, aprotynin and aspirin on trombolysis in cats.

pretreatment	n	percent change ± SD	
		fall in BP	thrombolysis
KALLIKREIN 5 U/kg	26	46 ± 17	34 ± 12
ASA 50 mg/kg+ KALLIKREIN 5 U/kg	14	34 ± 14	3 ± 2*
APROTYNIN 100.000 U/kg+ KALLIKREIN 5 U/kg	6	4 ± 3*	5 ± 3*
CAPTOPRIL 50 µg/kg+ KALLIKREIN 5 U/kg	6	53 ± 12	78 ± 10*
CAPTOPRIL 200 µg/kg	5	18 ± 6*	28 ± 9

* $p < 0.05$ vs kallikrein

aprotynin, but hardly influenced by ASA. Captopril at a high dose (200 μg/kg, i.v.) showed thrombolytic and hypotensive actions of its own (*Fig. 3, Table 1*). The thrombolytic action of captopril was completely inhibited by ASA. Captopril at a concentration of 2 μg/kg O.T. had no thrombolytic effect.

DISCUSSION

In our experiments the thrombolytic activity of kallikrein was assayed as dissipation of preformed blood clots in extracorporeal circulation. The thrombolytic action of kallikrein is associated with its enzymatic properties as evidenced by its inhibition with aprotynin, a kallikrein inhibitor (13). Therefore, the next link in the thrombolytic action of kallikrein comprises endogenous kinins. Indeed, the trombolytic action of kallikrein is enhanced by captopril - an ACE inhibitor (5) which is also responsible for inactivation of kinis (6). In turn, the thrombolytic action of endogenous kinins is mediated by prostacyclin as evidenced by the following. Fistly, the inhibition of cyclooxygenase by a high dose of ASA prevented the kallikrein-induced thrombolysis. Secondly, the lability of thrombolytic principle released by kallikrein was similar to that of synthetic or endogenous (i.e. released by nicotinamide) prostacyclin. Thirdly, kallikrein had no thrombolytic activity of its own when administered locally, over blood-superfused thrombi.

Bk is known to release prostacyclin along with EDRF(NO) from the vascular endothelium (14). Prostacyclin has platelet suppressant (15) fibrinolytic (16) and thrombolytic (17) properties. Although EDRF(NO) has been also claimed to inhibit platelet aggregation (18) and it is likely to be released *in vivo* (19), presently we have to assume that EDRF(NO) is not likely to participate in the mediation of the thrombolytic action of kallikrein since ASA completely prevents the thrombolytic action of kallikrein, while the release of EDRF(NO) is not influenced by inhibition of cyclooxygenase (20). There is, as yet no direct evidence to support this assumption.

In summary, we have demonstrated that kallikrein apart from its known vasodilator action has also thrombolytic properties. Both properties of kallikrein are kinin-mediated. Kinins do release prostacyclin and EDRF(NO) from vascular endothelium. The thrombolytic action of kallikrein seems to be mediated by prostacyclin. Captopril potentiates the thrombolytic potency of kallikrein. In fact captopril at high doses has thrombolytic action of its own. We propose that along with its vasodilator action thrombolytic properties of kallikrein are responsible for its therapeutical efficacy in peripheral vascular diseases (1, 2, 8).

Acknowledgement: We are very grateful to Dr G. Stock from Schering, Berlin and Dr G.: Varchmin from Bayropharm, Cologne for their generous gifts of iloprost and kallikrein (Depot-Padutin), respectively. Captopril (Iopril) was of Squibb Paris and prostacyclin sodium salt of Chinoin Budapest.

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Received: February 25, 1993
Accepted: March 1, 1993

Author's address: R. J. Gryglewski, Department of Pharmacology, N. Copernicus University School of Medicine, Grzegórzecka 16, 31-531 Cracow, Poland.