Kinetics of the platelet release reaction induced by collagen

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Collagen induces platelet aggregation and releases constituents from platelet granules, but the relationship between these responses has not been established. We have investigated this by measuring the release of 5-hydroxytryptamine (5-HT) and total adenine nucleotides from rat blood platelets over a period of 2 min after the addition of varying concentrations of collagen.

Platelet aggregation induced by collagen extract (CE) (Gordon & Gresham, 1972) was measured photometrically in rat citrated platelet-rich plasma. Adenine nucleotides (AN) and 5-HT in platelets and in supernatant plasma were measured fluorimetrically (Gordon & Drummond, 1974; Drummond & Gordon, 1974).

With a CE concentration (34 μg/ml) giving a maximal aggregation response there was progressive release of both AN and 5-HT (maximal after 2 minutes). With lower concentrations of CE, release of 5-HT and AN was divided into two kinetically distinct components. The first release occurred within 10 s of adding the CE, well before platelet aggregation began, and the second release parallelled the aggregation response. There was appreciable re-uptake of the initially released 5-HT before the second phase of release occurred (Figure 1). Platelet aggregation and the second phase of release were inhibited in parallel by EDTA (7.5 mM) and Colchicine (2.5 mM), but the initial release and the platelet shape change (which precedes aggregation) were unaffected. Prostaglandin E_1 (30 nM) and the pyrimido-pyrimidine derivative VK 774 (100 μM) inhibited both phases of release as well as the platelet shape change and the aggregation response.

Previous studies have failed to demonstrate two distinct phases of release with an intervening stage

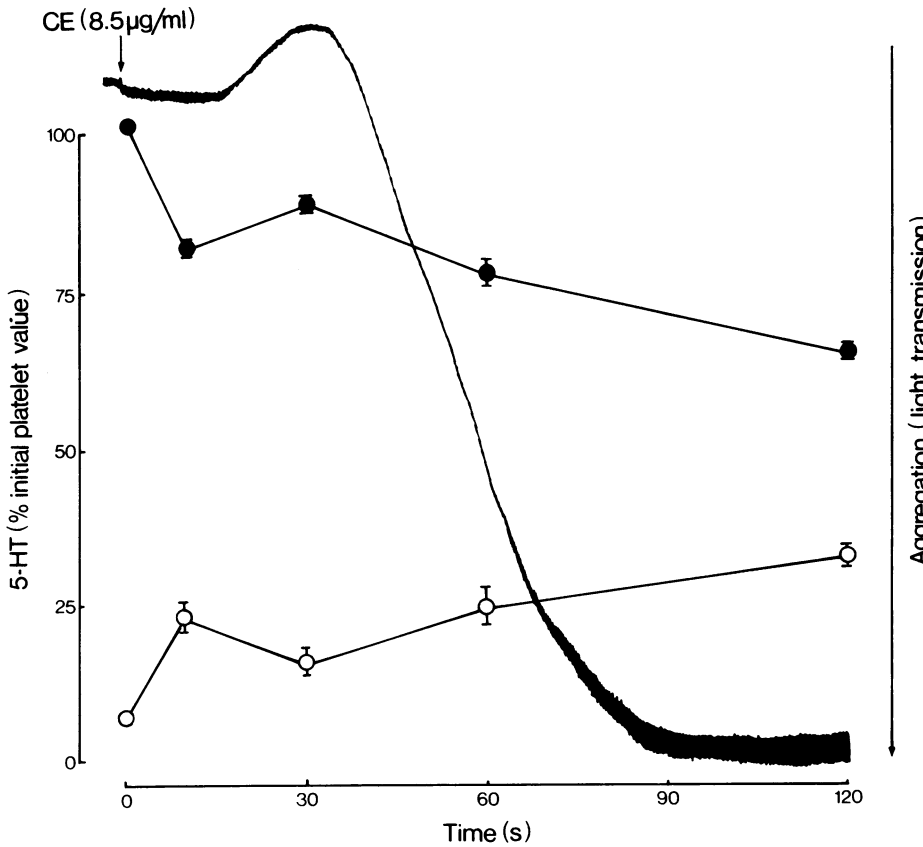

Fig. 1 Platelet aggregation and release of 5-hydroxytryptamine (5-HT) induced by collagen extract (CE). (•) Intracellular; (○) extracellular. Note initial release followed by partial reuptake and then secondary release. Points shown are means ± s.e. of six determinations. The content of 5-HT in platelets from control samples was 1.12 μg/10^9 cells.

of re-uptake (Packham, Guccione, Chang & Mustard, 1973; Weiss, Rogers & Brand, 1973). The reasons for this will be discussed. Our results support the concept that collagen induces platelet aggregation by releasing platelet constituents (Haslam, 1967). We suggest that the initial release is associated with platelet-collagen adhesion; both processes occur rapidly and are independent of extracellular calcium.

A.H.D. is an M.R.C. scholar.

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Receptors for 5-hydroxytryptamine and noradrenaline in rabbit aorta and central ear artery

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There is considerable evidence implicating 5-hydroxytryptamine (5-HT) in the vascular disturbances associated with migraine (Lance, Anthony & Hinterberger, 1970). As part of a study into the pharmacology of drugs used in the treatment of migraine we have examined the characteristics of 5-HT receptors in vascular smooth muscle from rabbit aorta and ear artery. In addition, the pharmacological characteristics of the α-adrenoceptors in these two tissues were examined.

Preparations were obtained from New Zealand white rabbits anaesthetized with pentobarbitone sodium (36 mg/kg i.v.). Isolated aortic strips and central ear arteries were set up as described by Furchgott & Bhadrakom (1953) and de la Lande & Rand (1965) respectively. Tissues were maintained in Krebs solution gassed with 5% CO_2 in O_2 at

Table 1 Interactions between agonists and antagonists in rabbit vascular smooth muscle

		pA_2 (30 min) against				
Artery	*Antagonist*	*5-HT*	*Methysergide*	*Noradrenaline*	*KCl*	*Vasopressin*
Aorta	Methysergide	8.49 (7.85-9.14)	–	5.29 (4.95-5.63)	<4.6	–
Aorta	Phentolamine	6.21 (5.52-6.90)	–	7.96 (7.76-8.16)	<4.6	–
Central ear	Pizotifen	6.79 (6.39-7.18)	6.95 (6.48-7.42)	6.57 (6.15-6.98)	–	<5.4
Central ear	Phentolamine	8.41 (7.99-8.83)	8.25 (7.96-8.54)	8.09 (7.54-8.64)	–	<7.3

In the aortic strip both agonists and antagonists were added to the bathing solution. In the ear artery the agonists were administered as a bolus injection close to the artery and antagonists added directly to the perfusion reservoir.

The primary and secondary responses to 5-HT in the central ear artery were antagonized to the same extent by pizotifen and phentolamine. The results for the primary phase are presented in Table 1 In the case of methysergide the primary phase was often poorly defined and only the secondary phase was examined.

Each value quoted is the mean (95% confidence limits) of 4-8 separate estimates.