Effect of Dinitrophenol and Glucose on Amine Release from Isolated Rabbit Blood Platelets

One of the results of anaphylaxis in the rabbit is the release of histamine and 5-hydroxytryptamine (serotonin) from platelets[1]. A role for a humoral agent in this process has been indicated by results[2] showing that fresh rabbit plasma is required for platelet amine release in *in vitro* antigen-antibody reactions. Anaphylatoxin, or a histamine releasing humoral factor similar to it, has been shown to arise in rabbit[2,3], rat[4], and guinea-pig[4] plasma incubated with immunoprecipitates or certain polysaccharides, and has been suggested as the humoral mediator of anaphylactoid states[5]. Previous work from this laboratory[6] had shown that histamine release from guinea pig lung incubated with agar anaphylatoxin did not take place in dinitrophenol treated tissue but occurred normally upon addition of glucose to the incubation medium. The present note indicates that a similar result is obtained when the release of histamine, as well as of serotonin, from rabbit platelets incubated with agar-activated rabbit plasma is examined in the presence of dinitrophenol and glucose.

Washed rabbit platelets were obtained from heparinized blood according to the technique described by HUMPHREY and JAQUES[2]. Rabbit plasma anaphylatoxin, activated in the manner described for rat plasma[7], was centrifuged to remove excess agar and dialysed in the cold against saline to remove glucose or other metabolites; like rat anaphylatoxin, it was found to remain active after dialysis. Amine release was induced by adding 0.25 ml of activated plasma to platelets suspended in gelatin-Tyrode medium[2] from which ascorbate and glucose were omitted; final volumes were 1.0 ml. Incubations were performed at 37°C for 15 min; where so indicated, a 15 min pre-incubation with dinitrophenol (DNP) and/or glucose preceded the addition of plasma. Silicone-treated glassware and low temperatures (2–4°C) were used for all preparative manipulations. The experiments were terminated by cooling the mixtures in crushed ice and separating the platelets by centrifugation in the cold; supernatants containing released amines were either assayed immediately or following storage at an acid pH. Residual histamine and serotonin were extracted from the sedimented platelets with hot 0.1 *N* HCl. Histamine was estimated on the atropinized guinea-pig ileum; serotonin was measured on the same preparation after blockade of its sensitivity to histamine with an antihistaminic[2].

Effect of DNP and glucose on the release of histamine and serotonin from isolated rabbit platelets incubated with agar-activated rabbit plasma

Incubation conditions	Histamine release (% ± S.E.)		Serotonin release (% ± S.E)	
Controls	67 ± 8	(5)	52 ± 12	(3)
Controls + glucose, 4.5 m*M*	61 ± 11	(5)	41 ± 6	(4)
DNP, 0.3 m*M*	34 ± 8	(5)	23 ± 13	(4)
DNP + glucose	80 ± 11	(5)	60 ± 2	(4)
DNP (no plasma)	26 ± 6	(5)	18 ± 7	(4)
DNP + glucose (no plasma)	30 ± 6	(5)	18 ± 6	(4)

Figures in parenthesis refer to the number of experiments performed; each of these was done with platelets from a different animal but not necessarily with plasma from different animals.

The results shown in the Table, in which amine release is expressed as percentage of total platelet histamine and serotonin liberated into the supernatant during incubation, indicate that approximately 40% of platelet aminēs are released by agar-anaphylatoxin; this release is not affected by glucose. Pre-treatment of the platelets with dinitrophenol (DNP), in a glucose-free medium, inhibited this release; in contrast, in the presence of the sugar, the effect of DNP was completely reversed. These results are compatible with observations showing that platelets contain the normal cellular ingredients for respiratory and glycolytic activity[8]. In view of the well known uncoupling effect of DNP on oxidative phosphorylation, glycolysis is probably responsible for the reactivating effect of glucose on platelet amine release in the DNP-treated system; this process apparently requires a supply of endogenous ATP and is, in this respect, similar to histamine release from tissues submitted to anaphylaxis[6,9] or the action of compound 48/80[10]. The pathway by which cell metabolism conditions amine release is not known; a merocrine secretory character has been postulated for the discharge of histamine from rat cells[11], and it is possible that such an activity could also lead to amine release from platelets. In this connection, it is perhaps pertinent to note observations by LANDSCHÜTZ[12], indicating that sensitized platelets possess a contractile ATP-requiring system which is activated following contact with specific antigen. Further studies will be required to show whether such a system could provide the explanation for the link between cellular metabolism and the release of vasoactive amines from platelet storage sites.

Zusammenfassung. Agar-aktiviertes Kaninchenplasma (Anaphylatoxin) setzt *in vitro* Histamin und Serotonin aus normalen Kaninchenblutplättchen frei. Dieser Effekt ist vom Stoffwechsel der Thrombocyten abhängig, da er durch Dinitrophenol gehemmt wird. Bei Anwesenheit von Glukose wird die Hemmung aufgehoben.

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