Table 2. Effect of gentamicin on the degradation of different size plasma protein fractions by MDCK cells in culture

Gentamicin concentration	Time of incubation	Degradation (%) Low mol. wt. fraction	Intermediate mol. wt. fraction	High mol. wt. fraction
None (control)	6	0.4 ± 0.09	1.2 ± 0.09	1.75 ± 0.2
	24	1.6 ± 0.06	3.8 ± 0.2	4.25 ± 0
250	6	0.3 ± 0.15	1.14 ± 0.1	0.85 ± 0.15
	24	1.74 ± 0.37	3.75 ± 0.3	4.13 ± 0.2
500	6	0.1 ± 0.03*	1.98 ± 0.04	1.65 ± 0.3
	24	1.25 ± 0.11*	2.75 ± 0.11*	4.20 ± 0.35
750	6	0.1 ± 0.04*	1.6 ± 0.3	1.7 ± 0.09
	24	1.42 ± 0.1*	2.05 ± 0.2*	4.5 ± 0.5
1000	6	0.13 ± 0.05*	1.09 ± 0.09	0.9 ± 0.2*
	24	1.39 ± 0.1*	1.75 ± 0.1*	1.68 ± 0.2*

Cells incubated in MEM containing 10% serum plus gentamicin for 5 days prior to experiment. Each value represents data from 4 replications ± SD. *For these values $P < 0.01$.

feedback on uptake. However, no clear indication of such an inhibition of degradation was observed in this study. Thus the prime target of gentamicin seems to be endocytosis though the mechanism may be direct or indirect. This inhibition of uptake in tubule cells may well contribute to the proteinuria found in aminoglycoside nephrotoxicity.

In summary, a kidney tubule cell line—MDCK cells—has been used to study the effects of gentamicin on the endocytosis of size fractionated plasma proteins by kidney tubule cells. Cells cultured in the presence of gentamicin for 5 days show reduced uptake and degradation compared to the corresponding control incubation. This inhibition by gentamicin may be attributed to its effect on endocytosis.

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Inositol phospholipid metabolism and platelet function*

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Platelet agonists of physiological or pathophysiological significance include von Willebrand factor, collagen, thrombin, ADP, platelet-activating factor, serotonin, prostaglandin endoperoxides, thromboxane A_2, vasopressin and epinephrine. They all bind to specific receptors on the platelet surface and trigger molecular events which finally lead to the various platelet responses, i.e. adhesion, shape change, release reaction and aggregation.

An early biochemical event upon platelet activation is the degradation of inositol phospholipids by phospholipase C resulting in the rapid formation of inositol trisphosphate, 1,2-diacylglycerol, and its phosphorylated product, phosphatidic acid [1–3]. These substances remain inside the platelets and act as mediators and/or modulators of platelet

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function. Specific links between the formation of those substances and subsequent molecular events are the activation of protein kinase C by diacylglycerol [4] and the mobilization of Ca^{2+} from the dense tubular system by inositol 1,4,5-trisphosphate [5]. Phosphatidylinositol 4,5-bisphosphate may—in addition to Mg^{2+}—contain Ca^{2+} bound to its negatively charged phosphate groups; its breakdown could then also lead to an increase of the cytosolic Ca^{2+} [6]. Whether phosphatidic acid and lysophosphatidic acid fulfill specific functions for platelet activation is at present unclear. The intracellular formation of phosphatidic acid and lysophosphatidic acid from exogenous 1,2-didecanoylglycerol does not evoke a rise of cytosolic Ca^{2+} [7], but it is followed by a slow aggregation response which may not solely be a consequence of protein kinase C activation induced by 1,2-didecanoylglycerol. In addition, diacylglycerol and phosphatidic acid could through physicochemical membrane changes influence platelet function: they enhance the fusion of membranes and may thereby facilitate platelet secretion [8, 9].

The present communication compares the effects of various platelet agonists on inositol phospholipid hydrolysis, intracellular Ca^{2+} mobilization and protein phosphorylation in relationship to specific responses of human platelets. The results indicate the existence of inositol phospholipid dependent and independent pathways for platelet activation.

The effects of various platelet agonists on inositol phospholipids, intracellular Ca^{2+} mobilization, protein phosphorylation and platelet responses are listed in Table 1. Only results of studies in which the observed effects are a direct consequence of the agonist with its receptor and not mediated by the formation of platelet endoperoxides and thromboxane A_2 or the release of ADP are shown.

It is obvious that the majority of platelet agonists induces inositol phospholipid hydrolysis, intracellular Ca^{2+} mobilization and protein phosphorylation. These changes occur in the absence of extracellular Ca^{2+} and Mg^{2+} [10–16] and are related to shape change and secretion (see Table 1).

Addition of thrombin, vasopressin, platelet-activating factor or prostaglandin endoperoxide analogs to platelets results within 5 secs in the formation of diacylglycerol, phosphatidic acid, inositol 1,4-bisphosphate and inositol 1,4,5-trisphosphate in human platelets [1–3, 10–13, 17–23]. The formation of inositol 1- and 4-monophosphate occurs after a time lag of 10–15 sec [23]. A small formation of inositol 1,2-cyclic monophosphate, which accounts for ~1% of the inositol monophosphates and occurs also after a time lag of 10–15 sec, could be detected in thrombin-stimulated platelets [24]. Therefore, polyphosphoinositides rather than phosphatidylinositol are initially hydrolyzed. The sequence of the early biochemical events may follow the model depicted in Fig. 1. The phosphorylated myosin might directly initiate alterations in the platelet cytoskeleton leading to shape change [14, 15]. The function of the 40k (47k) dalton protein phosphorylated by protein kinase C is not known.

Although many stimuli may initiate platelet activation according to this model (Fig. 1), some contradictions and exceptions exist which may indicate the existence of alternative pathways for platelet activation:

(a) ADP, platelet-activating factor and thrombin can evoke shape change, myosin light chain phosphorylation, secretion and aggregation at near basal levels of cytosolic Ca^{2+} which are well below the Ca^{2+} concentrations needed when Ca^{2+} ionophores are the stimuli. These results are against the concept that cytosolic Ca^{2+} alone is a trigger for platelet activation [16, 25–27].

(b) Collagen does not seem to induce Ca^{2+} mobilization

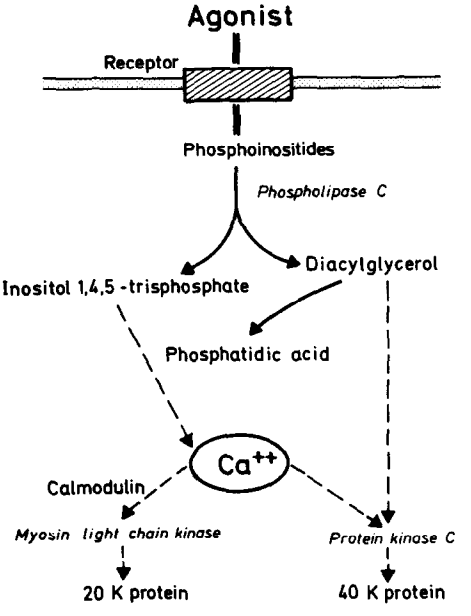

Fig. 1. Inositol phospholipid hydrolysis as signal to initiate platelet activation (reproduced with permission from [51]).

in aspirinized platelets [22, 25, 29], although it causes inositol phospholipid hydrolysis (including the formation of inositol trisphosphate) and protein phosphorylation [17, 22].

(c) ADP induces in the absence of extracellular Ca^{2+} shape change, a small increase of cytosolic Ca^{2+} and the phosphorylation of the 20k dalton myosin light chain but it does not evoke a measurable formation of inositol phosphates and diacylglycerol, a significant phosphorylation of the 47k dalton protein and a detectable degradation of phosphatidylinositol 4,5-bisphosphate in human platelets [14, 27–30]. A small increase of phosphatidic acid in human platelets stimulated by ADP can, however, be detected.*

(d) Studies using human platelets permeabilized with saponin, which are ATP-depleted, show that thrombin and collagen can evoke secretion and aggregation without stimulating inositol phospholipid hydrolysis and protein phosphorylation [31, 32].

(e) Epinephrine which binds to α_2-receptors induces the exposure of fibrinogen receptors and primary aggregation, but not the formation of inositol phosphates, diacylglycerol, and phosphatidic acid, the phosphorylation of the 47k dalton protein and Ca^{2+}-mobilization [12, 29, 30, 33, 34]. Platelet aggregation is thus independent of inositol phospholipid hydrolysis. This response requires the exposure of fibrinogen-receptors to which fibrinogen binds in the presence of extracellular Ca^{2+} or Mg^{2+} [35]. Therefore, the exposure of fibrinogen receptors which involves a Ca^{2+}-dependent conformational change of the glycoprotein IIb·IIIa complex [36] can be induced in the absence of phospholipase C activation. A further conclusion from the results with epinephrine is that activation of N_i (a regulatory GTP-binding protein that inhibits adenylate cyclase) by epinephrine does not cause phospholipase C activation [12].

In conclusion, beside a pathway involving inositol phospholipid hydrolysis, there may exist mechanisms for platelet activation which are independent of inositol phospholipids and are responsible for the exposure of fibrinogen receptors and platelet aggregation, for intracellular Ca^{2+} mobilization independent of inositol trisphosphate formation and for the phosphorylation of the 20k myosin light chain.

* W. Siess and E. Lapetina, unpublished observations.

Table 1. Effects of various platelet agonists on inositol phospholipid hydrolysis, intracellular Ca^{2+} mobilization, protein phosphorylation and responses of human platelets

Agonist	Inositol phospholipid hydrolysis: Diacylglycerol	Phosphatidic acid	Inositol-phosphates	Ca^{2+}-mobilization	Protein phosphorylation: 20k	47k	Platelet responses	References
Thrombin, 0.05–0.1 U/ml	+	+	+	+	+	+	Shape change	12, 16, 17, 19, 26, 37
>0.3 U/ml	+++	+++	+++	++	++	++	Secretion	1–3, 12, 16, 17–21, 29, 38
Collagen	+	+	+	−	+	+	Adhesion, secretion	17, 22, 25, 29, 39
Platelet-activating factor	+	+	+	+	+	+	Shape change	12, 26, 30, 40, 41
Vasopressin (V_1-receptor)	+	+	+	+			Shape change	13, 42
Arachidonic acid, 0.05–0.2 μM	+	+				+	Shape change	10
0.5–50 μM	++	++		+	++	++	Secretion	10, 43, 44
Dihomogammalinolenic acid, 2.5–20 μM	+	+				+	Shape change	45
Prostaglandin endoperoxide analogs	+	+	+	+	+	+	Shape change	11, 14, 22, 46
Serotonin (S_2-receptor)	+	+	+	+	+	+	Shape change	47–50
ADP	−	(+)	−	+	+	−	Shape change	14, 27–29, 30, 33
Epinephrine (α_2-receptor)	−	−	−	−		−	Primary aggregation	12, 29, 30, 33, 34

Only *direct* effects of the various agonists are listed. The effect of arachidonic acid is due to formation of endoperoxides and thromboxane A_2 [10]; the effect of dihomogammalinolenic acid is due to formation of endoperoxides [45]. Symbols indicate stimulation (+) or no changes (−).

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