Activation of V_1 -receptors by vasopressin stimulates inositol phospholipid hydrolysis and arachidonate metabolism in human platelets*

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The activation of platelet V_1 -receptors by vasopressin (0.01 – $1 \mu\text{M}$) induces the rapid formation of inositol phosphates, 1,2-diacylglycerol and phosphatidic acid, indicating inositol phospholipid hydrolysis by phospholipase C. Vasopressin immediately induces the formation of inositol bisphosphate and inositol trisphosphate. Accumulation of inositol 1-monophosphate and inositol 4-monophosphate occurs later after a time lag of 15 s. Low concentrations (10 – 100 nM) of vasopressin only activate phospholipase C, whereas high concentrations ($1 \mu\text{M}$) induce activation of phospholipase C and subsequently the production of arachidonate metabolites. Cyclo-oxygenase metabolites are associated with further activation of phospholipase C, release reaction and irreversible platelet aggregation. Vasopressin requires for its action extracellular Mg^{2+} , but not Ca^{2+} . The described platelet changes are not induced by 1-desamino-[8-D-arginine]vasopressin, a V_2 -receptor agonist, and are blocked by a specific V_1 -receptor antagonist. The results indicate that platelets possess a V_1 -receptor that is coupled to polyphosphoinositide hydrolysis by phospholipase C, leading to the formation of 1,2-diacylglycerol and inositol trisphosphate. Those compounds may act as second messengers for platelet responses induced by vasopressin, whereas endoperoxides and thromboxane A_2 stimulated by vasopressin may serve as amplifiers for platelet activation.

INTRODUCTION

Vasopressin induces the pressor and antidiuretic response through activation of two separate receptors, the V_1 - and V_2 -receptor respectively (Michell *et al.*, 1979). The receptors are coupled to different intracellular second messengers and differ in their molecular size (Michell *et al.*, 1979; Crause *et al.*, 1984). For example, V_1 -receptor activation in hepatocytes is related to inositol phospholipid hydrolysis and intracellular Ca^{2+} mobilization, whereas V_2 -receptor activation in renal tubular cells leads to an increase of cyclic AMP (Kirk *et al.*, 1977, 1979, 1981; Billah & Michell, 1979; Michell *et al.*, 1981; Thomas *et al.*, 1984; Charest *et al.*, 1985; Butlen *et al.*, 1978). In addition to phosphoinositide breakdown and cyclic AMP accumulation, vasopressin can also stimulate prostaglandin formation in various cell types (Hassid & Williams, 1983; Pfeilschifter *et al.*, 1984; Lieberthal & Levine, 1984; Ausiello & Zusman, 1984).

In platelets, vasopressin induces shape change, aggregation and release reaction, most probably through activation of V_1 -receptors (Haslam & Rosson, 1972; M. E. Thomas *et al.*, 1983). Although V_1 -receptor activation is coupled with an inhibition of adenylate cyclase activity in particulate fractions of platelets, vasopressin does not decrease cyclic AMP levels in intact platelets (Vanderwel *et al.*, 1983). Therefore second messengers, other than a decrease of cyclic AMP, may be involved in platelet activation induced by vasopressin. A wide variety of platelet agonists induces inositol phospholipid cleavage in human platelets by phospho-

lipase C (Lapetina & Cuatrecasas 1979; Rittenhouse-Simmons, 1979; Broekman *et al.*, 1980; Lapetina, 1982; Lapetina & Siess, 1983; Siess *et al.*, 1983a,b, 1984a,b, 1985) leading to two putative second messengers: 1,2-diacylglycerol activates protein kinase C; and inositol 1,4,5-trisphosphate mobilizes Ca^{2+} from the endoplasmic reticulum in various cell types (Nishizuka, 1983; Streb *et al.*, 1983; Berridge, 1984). Recently it has been observed that vasopressin increases cytoplasmic Ca^{2+} in human platelets partly by release from intracellular stores (Hallam *et al.*, 1984). Besides a preliminary report indicating that vasopressin stimulates the formation of phosphatidic acid in platelets (MacIntyre & Pollock, 1982) nothing is known about the effect of vasopressin on platelet phospholipid metabolism. We attempted, therefore, to study the action of vasopressin on platelet inositol phospholipid metabolism and on production of arachidonate metabolites in relation to specific platelet responses.

EXPERIMENTAL PROCEDURES

Materials

[Lysine]vasopressin was obtained from Sigma. 1-Desamino-[8-D-arginine]vasopressin (DDAVP, Minirin) was obtained from Ferring (Kiel, Germany). The V_1 -receptor antagonist D-(CH_2)₅Tyr(Me)-AVP ('Manning Compound') was purchased from Bachem (Torrance, CA, U.S.A.). [^3H]Inositol was obtained from New England Nuclear Corp. All other materials were obtained as described previously (Siess *et al.*, 1984b).

Abbreviations used: AVP, [8-D-arginine]vasopressin; DDAVP, 1-desamino-[8-D-arginine]vasopressin; PtdIns, phosphatidylinositol; PtdIns4P, phosphatidylinositol 4-phosphate; PtdIns(4,5) P_2 , phosphatidylinositol 4,5-bisphosphate; HHT, 12-hydroxyheptadeca-5,8,10-trienoic acid; 12-HETE, 12-hydroxyeicosa-5,8,10,14-tetraenoic acid.

* Dedicated to Professor Eberhard Buchborn on the occasion of his 65th birthday.

Measurement of platelet lipids

Platelet-rich plasma from 200 ml of human blood was incubated for 90 min at 37 °C with 400 μ Ci of [3 H]arachidonic acid in the presence of prostaglandin E₁ (1 μ g/ml). Platelets were then centrifuged, washed once with 30 ml of Tyrode/Hepes buffer containing CaCl₂ (0.1 mM), MgCl₂ (1 mM), heparin (25 units/ml), potato apyrase (200 μ g/ml) and prostacyclin (300 ng/ml), and finally resuspended in 20–25 ml of Tyrode/Hepes buffer containing CaCl₂ (0.1 mM), MgCl₂ (1 mM) and potato apyrase (200 μ g/ml) (Siess *et al.*, 1984b). Platelets were kept at 37 °C and the platelet concentration was adjusted to $(6-7) \times 10^8$ /ml. Experiments were carried out between 30 and 90 min after final resuspension. Platelet suspensions (1.5 ml) were placed into aggregometer tubes, stirred (1100 rev./min) for 3 min at 37 °C in the aggregometer and then exposed to vasopressin or DDAVP for various times. Light transmission was recorded and displayed on a chart recorder. Aliquots (0.2 ml) were transferred into 0.75 ml of chloroform/methanol (1:2, v/v), and 3 H-labelled platelet lipids were extracted, separated by thin-layer chromatography and measured as described previously (Siess *et al.*, 1984b).

Measurement of inositol phosphates

Platelet-rich plasma from 50 ml of blood was centrifuged after addition of prostacyclin (300 ng/ml) and the platelets were resuspended in 1 ml of Tyrode/Hepes

buffer containing 1 mM-MgCl₂, 1 mM-EGTA, apyrase (100 μ g/ml) and 2 mCi of [3 H]inositol. The platelet suspension [$(2-2.5) \times 10^9$ platelets/ml] was incubated at 37 °C for 3 h in aggregometer tubes while being stirred (250 rev./min). Prostacyclin (500 ng/ml) was added, and platelets were washed once in 8 ml of Tyrode/Hepes buffer containing inositol (5 mM), CaCl₂ (0.1 mM), MgCl₂ (1 mM), heparin (25 units/ml), apyrase (200 μ g/ml) and prostacyclin (300 ng/ml). After centrifugation, platelets were resuspended in 1.5–2 ml of Tyrode/Hepes buffer containing CaCl₂ (0.1 mM), MgCl₂ (1 mM) and apyrase (200 μ g/ml). The platelet concentration was adjusted to $(1-1.3) \times 10^9$ /ml. The platelet suspension was kept at 37 °C for 30–45 min to reach equilibrium between the free [3 H]inositol and the [3 H]inositol incorporated into platelet phosphoinositides. Under those conditions the proportions of radiolabelled PtdIns, PtdIns4P and PtdIns(4,5)P₂ obtained were $22.7 \pm 6:3.3 \pm 0.8:1$ (mean $\pm$ S.D., $n=7$). Unlabelled phosphoinositides in platelets show similar proportions (Mauco *et al.*, 1984). The specific radioactivity is thus equal for each radiolabelled platelet phosphoinositide and ranged between 15×10^6 and 100×10^6 c.p.m. of 3 H/ μ mol of phosphoinositide in our experiments.

Samples of platelet suspension (0.8 ml) were placed into aggregometer tubes, LiCl (10 mM) was added and platelets were stirred for 3 min before exposure to vasopressin or DDAVP. Aliquots (0.1 ml) were transferred at various times into 0.375 ml of chloroform/

Fig. 1. Aggregation tracings of human platelets stimulated by vasopressin (VP)

Platelets prelabelled with [3 H]inositol (Figs 1a–1c) or [3 H]arachidonic acid (Figs 1d–1f) were resuspended in Tyrode/Hepes buffer containing MgCl₂ (1 mM), CaCl₂ (0.1 mM) and apyrase (200 μ g/ml), and exposed to vasopressin (1 μ M) (a), DDAVP (1 μ M) (b), the V₁-receptor antagonist D-(CH₂)₅Tyr(Me)-AVP (0.1 μ M) plus vasopressin (1 μ M) (c), vasopressin (1 μ M) (d), citrate (22 mM) plus EGTA (5 mM) plus vasopressin (1 μ M) (e), citrate (22 mM) plus EDTA (5 mM) plus vasopressin (1 μ M) (f). Note that cells prelabelled with [3 H]inositol respond to 1 μ M-vasopressin with a reversible aggregation (a), whereas platelets prelabelled with [3 H]arachidonic acid respond with an irreversible aggregation (d).

methanol/conc. HCl (100:200:2, by vol.). After addition of a mixture of unlabelled glycerophosphoinositol, inositol 1-phosphate, inositol 2-phosphate, inositol 1,4-bisphosphate and inositol 1,4,5-trisphosphate, which were prepared as described recently (Siess & Binder, 1985; Binder *et al.*, 1985), the aqueous soluble inositol phosphates were extracted (Siess & Binder, 1985) and the samples were freeze-dried. The inositol phosphates were then separated by h.p.l.c. as described recently (Binder *et al.*, 1985). fractions were collected, split into two halves and measured for ^3H radioactivity or for phosphorus (Bartlett, 1959). Radioactive inositol phospholipids were determined in separate samples by using extraction at acidic pH and 2 M-KCl (Billah & Lapetina, 1982). They were separated on thin-layer plates impregnated with 1% potassium oxalate (Jolles *et al.*, 1981).

RESULTS

Vasopressin stimulates the formation of inositol phosphates

Human platelets prelabelled with [^3H]inositol were exposed to vasopressin ($1\ \mu\text{M}$), which induces shape change and reversible aggregation (Fig. 1a). Vasopressin stimulates the formation of four [^3H]inositol-labelled compounds that co-chromatograph on h.p.l.c. with unlabelled inositol 1-monophosphate, inositol 2-monophosphate, inositol 1,4-bisphosphate and inositol 1,4,5-trisphosphate (Fig. 2). Since in several experiments it was observed that inositol 2-monophosphate did not co-chromatograph exactly with the [^3H]inositol-labelled compound formed in vasopressin-stimulated platelets (see also Fig. 2c), a different h.p.l.c. system was set up to separate the following three inositol monophosphates: inositol 1-, inositol 2- and inositol 4-monophosphate. It was found that, in that system, the radioactive inositol monophosphates in control and vasopressin-stimulated platelets are co-eluted with inositol 1-monophosphate and inositol 4-monophosphate respectively (Fig. 3).

The formation of inositol bisphosphate and inositol trisphosphate is detectable 5 s after addition of vasopressin, whereas inositol 1-monophosphate and inositol 4-monophosphate increase after a time lag of 15 s (Figs. 2 and 4). The formation of inositol bisphosphate and inositol trisphosphate peaks at 30 s and then decreases. In contrast, inositol 1-monophosphate and inositol 4-monophosphate accumulate continuously during the time period tested (Fig. 4).

Vasopressin stimulates the formation of 1,2-diacylglycerol and phosphatidic acid, and the production of arachidonate metabolites

Human platelets prelabelled with [^3H]arachidonic acid were exposed to vasopressin ($1\ \mu\text{M}$), which induces release reaction and irreversible aggregation (Fig. 1d). Vasopressin stimulates the rapid formation of 1,2-diacylglycerol and phosphatidic acid. 1,2-Diacylglycerol peaks before phosphatidic acid, indicating the sequential activation of phospholipase C and diacylglycerol kinase. Free arachidonic acid also increases immediately, but the formation of cyclo-oxygenase metabolites such as HHT and thromboxane B_2 is delayed by 20 s. Metabolism by lipoxygenase to 12-HETE occurs even at later time points (Fig. 5).

Fig. 2. H.p.l.c. separation of inositol phosphates formed in human platelets after exposure to vasopressin (VP)

Human platelets prelabelled with [^3H]inositol were either exposed to saline (a) or to vasopressin ($1\ \mu\text{M}$) for 5 s (b) or 60 s (c). Aliquots (0.2 ml) of the platelet suspension were transferred into chloroform/methanol/HCl (Billah & Lapetina, 1982), and a mixture of authentic unlabelled glycerophosphoinositol and inositol phosphates was added. The samples were extracted, separated by h.p.l.c. (Binder *et al.*, 1985), and the fractions were measured for [^3H]radioactivity ($\bullet$) and for phosphorus ($\circ$). Incorporation of [^3H]inositol into phosphoinositides of 0.2 ml of platelet suspension was in this experiment 148 400 c.p.m. for PtdIns, 29 400 c.p.m. for PtdIns4P and 12 390 c.p.m. for PtdIns(4,5)P₂. The recovery of the added standard compounds after extraction and h.p.l.c. was $39 \pm 6\%$ for glycerophosphoinositol (GroPIns), $69 \pm 8\%$ for inositol 1-phosphate (Ins1P), $71 \pm 8\%$ for inositol 2-phosphate (Ins2P), $67 \pm 9\%$ for inositol 1,4-bisphosphate [Ins(1,4)P₂] and $51 \pm 1\%$ for inositol 1,4,5-trisphosphate [Ins(1,4,5)P₃]. The abbreviations in the upper panel (a) indicate the elution positions on h.p.l.c. of the added unlabelled standard compounds ($\circ$). Only purified standard substances of defined chemical structure have been used. They were prepared as described by Siess & Binder (1985) and Binder *et al.* (1985). Note the reduced scale of the left ordinate in (c).

Fig. 3. H.p.l.c. separation of inositol monophosphates formed in platelets after exposure to vasopressin (VP)

Human platelets prelabelled with [^3H]inositol were either exposed to saline (a) or to vasopressin ($1\ \mu\text{M}$) for 2 min (b). Aliquots (0.2 ml) of the platelet suspension were transferred into chloroform/methanol/HCl and a mixture of unlabelled inositol 1-monophosphate, inositol 2-monophosphate and inositol 4-monophosphate prepared as described recently (Siess, 1985) was added. The samples were extracted and purified by Dowex anion-exchange chromatography (Berridge, 1983). The fraction containing the inositol monophosphates was passed through Dowex HCR-W2 columns (Siess & Binder, 1985) and separated by h.p.l.c. on a freshly conditioned LiChrosorb NH_2 column (Merck, Darmstadt, Germany) as described recently (Siess, 1985). Separation was carried out by utilizing a 20 min isocratic elution with 50 mM-ammonium acetate/acetic acid buffer, pH 4.0, followed by a 60 min linear gradient to 1 M-ammonium acetate/acetic acid, pH 4.0, at a flow rate of 1 ml/min. Fractions were collected every 0.5 min and measured for radioactivity (●) and for phosphorus (○). Abbreviations are defined in Fig. 2.

Low concentrations of vasopressin (10–100 nM) induce platelet shape change and reversible platelet aggregation without release reaction (results not shown). These concentrations only stimulate phospholipase C, as indicated by the formation of 1,2-diacylglycerol and phosphatidic acid, without increasing the formation of arachidonate metabolites (Fig. 6). Only concentrations of vasopressin ($1\ \mu\text{M}$) that induce release reaction and irreversible aggregation stimulate both phospholipase C and the production of arachidonate metabolites (Figs. 1d and 6).

Fig. 4. Time course of formation of inositol phosphates in human platelets stimulated by vasopressin

Suspensions of human platelets [0.8 ml; $(1\text{--}1.3 \times 10^9$ platelets/ml)] prelabelled with [^3H]inositol were exposed to vasopressin ($1\ \mu\text{M}$) for various times. Aliquots (0.1 ml) were transferred into chloroform/methanol/HCl, a mixture of unlabeled inositol phosphate standards was added, two samples were pooled, and [^3H]labelled inositol phosphates were measured after extraction and separation by h.p.l.c. as shown in Fig. 2. The radioactivity of each phosphate ester was corrected to a standard incorporation of 10^2 d.p.m. of [^3H]inositol into the respective platelet inositol phospholipids. The values are means $\pm$ S.E.M. ($n = 3$). For abbreviations, see Fig. 2.

Effect of cyclo-oxygenase and lipoxygenase inhibition, and influence of extracellular bivalent cations on the action of vasopressin on platelets

Platelets that only respond with shape change and reversible aggregation after exposure to vasopressin do not produce arachidonate metabolites (Fig. 6). Therefore indomethacin does not change the formation of inositol phosphates in platelets that are prelabelled with [^3H]inositol and stimulated with $1\ \mu\text{M}$ -vasopressin (Fig. 1a, Table 1).

Similarly, indomethacin does not alter the production of phosphatidic acid in platelets that are prelabelled with [^3H]arachidonic acid and stimulated with low concentrations (10–100 nM) of vasopressin (results not shown). In experiments, however, in which vasopressin ($1\ \mu\text{M}$) induces the production of arachidonate metabolites and irreversible aggregation (Fig. 6), indomethacin (which inhibits cyclo-oxygenase) or BW 755 C (which inhibits both cyclo-oxygenase and lipoxygenase) causes a reduction of the phosphatidic acid formation at later time

Fig. 5. Effect of vasopressin (●) and DDAVP (○) on the formation of 1,2-diacylglycerol (1,2-DG) and phosphatidic acid (PA), the release of arachidonic acid (AA) and the production of arachidonate metabolites

Samples of platelet suspension (1.6 ml) prelabelled with [^3H]arachidonic acid were exposed to either 1 μM -vasopressin (●) or DDAVP (○). Aliquots (0.2 ml) were transferred at various times into chloroform/methanol and ^3H radioactivity in platelet lipids was measured (Siess *et al.*, 1984b). Values are means $\pm$ S.E.M. from four experiments. Further abbreviation: TXB₂, thromboxane B₂.

points; the initial phospholipase C activation is, however, not affected (Fig. 7). In the presence of those inhibitors, release of serotonin does not occur and only reversible platelet aggregation is observed (results not shown).

The action of vasopressin on platelets is critically dependent on the presence of extracellular Mg^{2+} . Mg^{2+} is needed for the shape-change and lipid responses induced by vasopressin, since chelation of Mg^{2+} abolishes completely the effect of vasopressin on platelet lipids and platelet responses (Fig. 1f, Table 2). Ca^{2+} is only required for the aggregation response, because the selective removal of extracellular Ca^{2+} only eliminates platelet

aggregation, but does not significantly affect platelet shape change and lipid responses induced by vasopressin (Fig. 1e, Table 2).

Vasopressin induces platelet inositol phospholipid hydrolysis through activation of V_1 -receptors

Preincubation of platelets with a 100 nM concentration of the specific V_1 -receptor antagonist D-(CH₂)₅Tyr(Me)-AVP (Sawyer & Manning, 1984) blocks completely the effect of vasopressin on platelet responses and inositol phosphate formation (Fig. 1c, Table 1). The V_1 -receptor antagonist inhibits dose-dependently the formation of

Fig. 6. Effects of various concentrations of vasopressin on phospholipase C activation and arachidonic acid metabolism in human platelets

Platelets prelabelled with [³H]arachidonic acid were exposed to vasopressin. 1,2-Diacylglycerol was measured 10 s after addition of vasopressin; phosphatidic acid and arachidonic acid were measured 30 s, and arachidonate metabolites, 60 s after addition of vasopressin. Values are means ± S.E.M. from six experiments. The asterisk (*) indicates irreversible platelet aggregation and release of [¹⁴C]serotonin (Siess *et al.*, 1983a) induced by 1 μM-vasopressin. For abbreviations see the legend to Fig. 5.

Fig. 7. Effects of indomethacin and BW755C on phosphatidic acid formation and arachidonic acid metabolism stimulated by vasopressin

Human platelets prelabelled with [³H]arachidonic acid were preincubated for 3 min at 37 °C with 0.1% ethanol (●), indomethacin (10 μM) (○) or BW 755C 100 μM (△), the latter two both dissolved in ethanol, before the addition of vasopressin (1 μM). For further details, see the legend to Fig. 5.

phosphatidic acid in human platelets stimulated by 1 μM-vasopressin (Fig. 8). In addition, the vasopressin analogue DDAVP, which activates V₂-receptors, does not induce platelet responses or the formation of inositol phosphates (Table 1, Fig. 1b). It also does not significantly enhance the formation of 1,2-diacylglycerol,

Table 1. Effect of DDAVP on the formation of inositol phosphates, and of indomethacin or a V₁-receptor antagonist on the vasopressin-induced formation of inositol phosphates in human platelets

Human platelets prelabelled with [³H]inositol were exposed to vasopressin or DDAVP for 1 min. The V₁-receptor antagonist D-(CH₂)₅Tyr(Me)-AVP or indomethacin was added 3 min before vasopressin. Radioactive inositol phosphates were measured after h.p.l.c. as indicated in Fig. 2. The Table shows replicate values from one out of three different experiments which gave similar results.

Additions	³ H radioactivity (d.p.m.)				
	Glycero-phosphoinositol	Inositol 1-phosphate	Inositol 4-phosphate	Inositol 1,4-bisphosphate	Inositol 1,4,5-trisphosphate
None	2023	869	669	228	77
	2234	880	763	209	100
Vasopressin (1 μM)	1958	2156	2189	1380	507
	2654	1915	2010	1176	555
Vasopressin (1 μM) + indomethacin (10 μM)	2302	2019	2056	1183	486
	1995	2134	1804	1109	455
Vasopressin (1 μM) + V ₁ -receptor antagonist (100 nM)	2615	858	747	245	39
	2091	904	856	228	100
DDAVP (1 μM)	2239	747	634	235	47
	2041	856	747	180	58

Table 2. Effect of chelation of extracellular Ca^{2+} and Mg^{2+} on the action of vasopressin on platelet lipids

Samples (1.0 ml) of human platelets prelabelled with [^3H]arachidonic acid were resuspended in buffer containing Ca^{2+} (0.1 mM) and Mg^{2+} (1 mM) and stimulated with vasopressin (1 μM) for 1 min. For chelation of extracellular Ca^{2+} , citrate and EGTA were added 3 min before vasopressin. For chelation of Ca^{2+} and Mg^{2+} , citrate and EDTA were added 3 min before vasopressin. Results are expressed as percentages of control (no addition of vasopressin) from three experiments (mean $\pm$ s.d.). Further abbreviations: PA, phosphatidic acid; TXB_2 , thromboxane B_2 .

Additions	PA	TXB_2	HHT	HETE	Platelet aggregation (% light transmission)
Vasopressin buffer	342 $\pm$ 62	240 $\pm$ 60	230 $\pm$ 9	193 $\pm$ 28	68 $\pm$ 10
Vasopressin + citrate (22 mM)	239 $\pm$ 19	178 $\pm$ 49	212 $\pm$ 67	192 $\pm$ 35	15 $\pm$ 2
Vasopressin + citrate (22 mM) + EGTA (5 mM)	279 $\pm$ 35	245 $\pm$ 55	203 $\pm$ 28	207 $\pm$ 42	Shape change
Vasopressin + citrate (22 mM) + EDTA (5 mM)	117 $\pm$ 12	106 $\pm$ 5	98 $\pm$ 17	117 $\pm$ 14	No activation

Fig. 8. Effects of various concentrations of the V_1 -receptor antagonist $\text{D}-(\text{CH}_2)_5\text{Tyr}(\text{Me})\text{-AVP}$ on the formation of phosphatidic acid induced by vasopressin

Human platelets prelabelled with [^3H]arachidonic acid were incubated with various concentrations of $\text{D}-(\text{CH}_2)_5\text{Tyr}(\text{Me})\text{-AVP}$ for 2 min at 37 $^\circ\text{C}$ before incubation with vasopressin (1 μM) for 1 min. Values are means $\pm$ S.E.M. from triplicate assays of two experiments from different platelet donors (O, ●).

phosphatidic acid, arachidonic acid and arachidonate metabolites (Fig. 5).

DISCUSSION

The effect of vasopressin on inositol phospholipids has been studied mainly in hepatocytes (Kirk *et al.*, 1977; Kirk *et al.*, 1979; Billah & Michell, 1979; Michell *et al.*, 1979, 1981; Kirk *et al.*, 1981; Prpic *et al.*, 1982; Thomas *et al.*, 1983, 1984; Litosch *et al.*, 1983; Seyfred & Wells, 1984; Hughes *et al.*, 1984; Charest *et al.*, 1985). Other tissues studied in which activation of V_1 -receptors leads to the stimulation of inositol phospholipid metabolism

include sympathetic ganglia (Bone *et al.*, 1984) and aorta strips (Takhar & Kirk, 1981). Our results indicate that, in platelets also, the activation of V_1 -receptors is coupled to inositol phospholipid hydrolysis by phospholipase C. Furthermore, V_1 -receptor activation in platelets leads to release of arachidonic acid and production of arachidonate metabolites. The formation of inositol trisphosphate and diacylglycerol occurs immediately after platelet exposure to vasopressin, and those compounds are therefore formed rapidly enough to act as second messengers for platelet activation.

Inositol bisphosphate and inositol trisphosphate are formed before inositol monophosphates increase, indicating rapid hydrolysis of polyphosphoinositides. The formation of inositol bis- and tris-phosphate rapidly decreases thereafter, whereas inositol monophosphates continuously accumulate. These results may indicate that inositol monophosphates are partly produced by degradation of inositol tris- and bis-phosphates by specific phosphomonoesterases (Downes *et al.*, 1982; Storey *et al.*, 1984). Breakdown of inositol 1,4-bisphosphate by phosphatases would lead to a mixture of inositol 1-monophosphate and inositol 4-monophosphate. We observed that, in fact, two radioactive inositol monophosphates are formed in vasopressin-stimulated platelets, which co-chromatograph with inositol 1-monophosphate and inositol 4-monophosphate. The two inositol monophosphates are formed in almost equal amounts, which may indicate that the phosphatases remove the 1- and 4-phosphate groups of inositol 1,4-bisphosphate at approximately similar rates. This suggestion is supported by the observation that the soluble phosphatase activity from liver does not show a precise positional selectivity for inositol 1,4-bisphosphate (Storey *et al.*, 1984). These data could be an indication that inositol monophosphates formed in vasopressin-stimulated platelets, in contrast with platelets stimulated by thrombin (Siess, 1985), are entirely formed from inositol bisphosphate; they do, however, not rule out the possibility that phospholipase C-induced hydrolysis of PtdIns also occurs.

The results may indicate that both $\text{PtdIns}(4,5)\text{P}_2$ and $\text{PtdIns}(4,5)\text{P}_3$ are degraded initially by phospholipase C in platelets stimulated by vasopressin. It can, however, not be excluded that only $\text{PtdIns}(4,5)\text{P}_2$ is degraded

initially and that inositol 1,4-bisphosphate is formed from inositol 1,4,5-trisphosphate instead of from PtdIns4P. Support for this pathway comes from the observation that ^{32}P -labelled PtdIns4P does not decrease after platelet activation with thrombin or endoperoxide analogue (Billah & Lapetina, 1982; Rittenhouse, 1984). Formation of inositol polyphosphates before accumulation of inositol monophosphates has also been observed recently for hepatocytes stimulated by vasopressin (Thomas *et al.*, 1984) and for platelets stimulated by thrombin, collagen and endoperoxide analogues (Watson *et al.*, 1984; Vickers *et al.*, 1984; Siess, 1985; Siess & Binder, 1985; Watson *et al.*, 1985).

In addition to inositol phospholipid hydrolysis by phospholipase C, vasopressin also induces release of arachidonic acid from membrane phospholipids and the production of arachidonate metabolites in human platelets. The formation of arachidonate metabolites occurs subsequently to phospholipase C activation. Cyclo-oxygenase metabolites such as endoperoxides and thromboxane A_2 are responsible for further activation of phospholipase C, induction of release reaction and irreversible platelet aggregation, since cyclo-oxygenase inhibition reduces phosphatidic acid formation, inhibits release reaction and results in reversible platelet aggregation induced by vasopressin. Thus cyclo-oxygenase products play a role in amplifying platelet responses through phospholipase C activation (Siess *et al.*, 1983a,b; Pollock *et al.*, 1984; Siess *et al.*, 1985; Watson *et al.*, 1985). Low concentrations of vasopressin (10–100 nM) only induce phosphoinositide hydrolysis by phospholipase C and release of arachidonic acid without the production of arachidonate metabolites. A similar dissociation of phospholipase C activation from the production of arachidonate metabolites has been observed previously in platelets stimulated by low concentrations of thrombin, platelet activating factor and prostaglandin endoperoxide analogues (Siess *et al.*, 1984b, 1985), and recently in fibroblasts stimulated by serum or bradykinin (Vicentini & Villereal, 1984).

Inositol phospholipid hydrolysis, release of arachidonic acid and production of arachidonate metabolites in human platelets stimulated by vasopressin are mediated by the activation of V_1 -receptor. DDAVP, a V_2 -receptor agonist, does not induce these responses, and the observed platelet changes by vasopressin are blocked by a specific V_1 -receptor antagonist. Our present findings indicate, therefore, that the V_1 -receptor present in human platelets is coupled to inositol phospholipid hydrolysis, release of arachidonic acid and production of arachidonate metabolites, and resembles the V_1 -receptors found in hepatocytes and vascular smooth muscles.

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