Acknowledgments. The authors are grateful to Prof. A. Iwashima for suggestions and helpful discussion. Thanks are also due to Kenji Sempuku for critically reading the manuscript.

1 Askari, A., J. gen. Physiol. *49* (1966) 1147.
2 Martin, K., J. gen. Physiol. *51* (1968) 497.
3 Diamond, I., and Kennedy, E. P., J. biol. Chem. *244* (1969) 3258.
4 Schuberth, A., Sundwall, B., Sorbo, B., and Lindell, J.-O., J. Neurochem. *13* (1966) 347.
5 Marchbanks, R. M., Biochem. J. *110* (1968) 533.
6 Plagemann, P. G. W., J. Lipid Res. *12* (1971) 715.
7 Adamic, S., Biochim. biophys. Acta *196* (1970) 113.
8 Sung, C.-P., and Johnstone, R. M., Can. J. Biochem. *43* (1965) 1111.
9 Sung, C.-P., and Johnstone, R. M., Biochim. biophys. Acta *173* (1969) 548.
10 Rennick, B. R., J. Pharmac. exp. Ther. *122* (1958) 449.
11 Acara, M., and Rennick, B. R., J. Pharmac. exp. Ther. *182* (1972) 1.
12 Herzerg, G. R., and Lerner, J., Biochim. biophys. Acta *307* (1973) 234.
13 Tuma, D. J., Barak, A. J., Schafer, D. F., and Sorell, M. F., Can. J. Biochem. *51* (1973) 117.
14 Zeisel, S. H., Story, D. L., Wurtman, R. J., and Brunengraber, H., Proc. natl Acad. Sci. USA *77* (1980) 4417.
15 Yoshioka, K., Nishimura, H., Himukai, M., and Iwashima, A., Biochim. biophys. Acta *815* (1985) 499.
16 Yoshioka, K., Biochim. biophys. Acta *778* (1984) 201.
17 Seglen, P. O., Exp. Cell Res. *74* (1975) 450.
18 Yoshioka, K., Nishimura, H., and Iwashima, A., Biochim. biophys. Acta *732* (1983) 308.
19 Whittaker, V. P., Handb. exp. Pharmak. *15* (1963) 23.
20 Potter, L. T., and Murphy, W., Biochem. Pharmac. *16* (1967) 1386.
21 Wilkinson, G. N., Biochem. J. *80* (1961) 324.

0014-4754/88/100889-04$1.50 + 0.20/0

In vitro enhancement of human platelet aggregation by somatostatin

P. P. Gazzaniga, G. Di Macco*, R. La Mancusa, A. Oddi*, G. Pappalardo*, F. M. Pulcinelli and D. Reggio*

*Department of Experimental Medicine, *Institute of 2nd Surgical Clinic, University of Rome La Sapienza, Rome (Italy)*
Received 27 November 1987; accepted 16 June 1988

Summary. Very low concentrations of somatostatin (S-14) strongly potentiate the in vitro aggregation induced by collagen, ristocetin and arachidonic acid, but not that induced by ADP or epinephrine, in both human platelet rich plasmas and gel-filtered platelet preparations. Desensitization phenomena may be induced either by repeated addition of S-14 or long lasting contact between S-14 and platelets.
Key words. Somatostatin; platelet aggregation; platelet activation.

The involvement of somatostatin in hemostasis and blood clotting has been extensively investigated [1-7], but its role in these physiological mechanisms is still largely obscure. While clotting factors appear to be unaffected by somatostatin both in animals and humans, thrombocytopenia has been reported in chronically somatostatin-treated baboons [8] and 24 h after somatostatin infusion in man [5,6], in spite of a lack of changes in platelet survival [8]. Inhibition of platelet aggregation after in vivo infusion in man and rabbits has been reported by some investigators [8-10]; however, no significant change has been reported under the same conditions in both platelet adhesion and aggregation [11]. The production of circulating platelet aggregates was enhanced after infusion of somatostatin in diabetic, but not in normal subjects [12]. In vitro studies also led to conflicting results as regards the effects of somatostatin on platelet function: evidence has been provided of a marked inhibition of platelet aggregation induced by ADP, collagen and ristocetin, in the presence of very high concentrations of somatostatin [8], whereas increased responses to epinephrine have been reported by others [9,12].

Some information has been obtained in recent years about platelet receptors for polypeptide hormones such as vasopressin [13-17]; specific recognition sites for somatostatin have been identified in several cell types, but not in human platelets [18]. Nevertheless, in this communication evidence will be given of a potentiating effect of very low concentrations of S-14 on the in vitro human platelet aggregation selectively induced by some platelet agonists.

Samples of platelet-rich plasma (PRP) and platelet-poor plasma (PPP) were obtained from healthy aspirin-free students of the Rome University Medical School. In some experiments we used, instead of PRP, suspensions of plasmaprotein-free platelets, obtained by gel filtration according to Tangen and Berman [19]: these gel-filtered platelets (GFP) showed normal ultrastructure, with uniform distribution of granules and absence of pseudopodia or other signs of activation. These GFPs, resuspended in phosphate buffer, aggregated in response to thrombin (> 0.05 U/ml), arachidonic acid (> 50 μM) and ristocetin (> 1.5 mg/ml) also in absence of added human fibrinogen and Ca^{2+}; to elicit a response to ADP (> 0.8 μM), epinephrine (> 5 μM) and collagen (> 4 μg/ml), the addition of human fibrinogen (final concentration 1.5 mg/ml) and Ca^{2+} (final concentration 25 μg/ml) were required.

The aggregation assays were performed in plastic cells, using a Dual Channel Elvi-Logos 840 Aggregometer. ADP, epinephrine, collagen, ristocetin and arachidonic acid were used as inducers. For each specimen of PRP or GFP, we sought the concentration of each of the aggregating agents which was able to induce a minimal primary aggregation wave (threshold concentration): then concentrations as low as at least 1/5 of the threshold ones were added to PRP or GFP. Prior to the addition of each aggregation inducer, somatostatin was introduced in one of the two cells; its final concentration was in different experiments 250 pg/ml, 1 ng/ml, 60 ng/ml, 300 ng/ml, 1 μg/ml. The second cell was used as a control. The interval between addition of somatostatin and that of the aggregation inducer ranged from 5 s to 20 min. In some experiments a second addition of the same dose of somatostatin followed the first one in the same cell containing PRP or GFP, 20 min or more later.

Somatostatin alone did not induce platelet aggregation, either in PRP or in GFP. After addition of somatostatin to PRP, subthreshold concentrations of collagen (from 0.1 to 2 μg/ml), arachidonic acid (from 0.55 to 1.1 μM) and ristocetin (from 0.5 to 1 mg/ml) induced a nearly maximal aggregation response (fig. 1). The table analytically reports the experiments performed at a concentration of 60 ng/ml (see also figs 1 and 2); however, S-14 showed similar enhancing

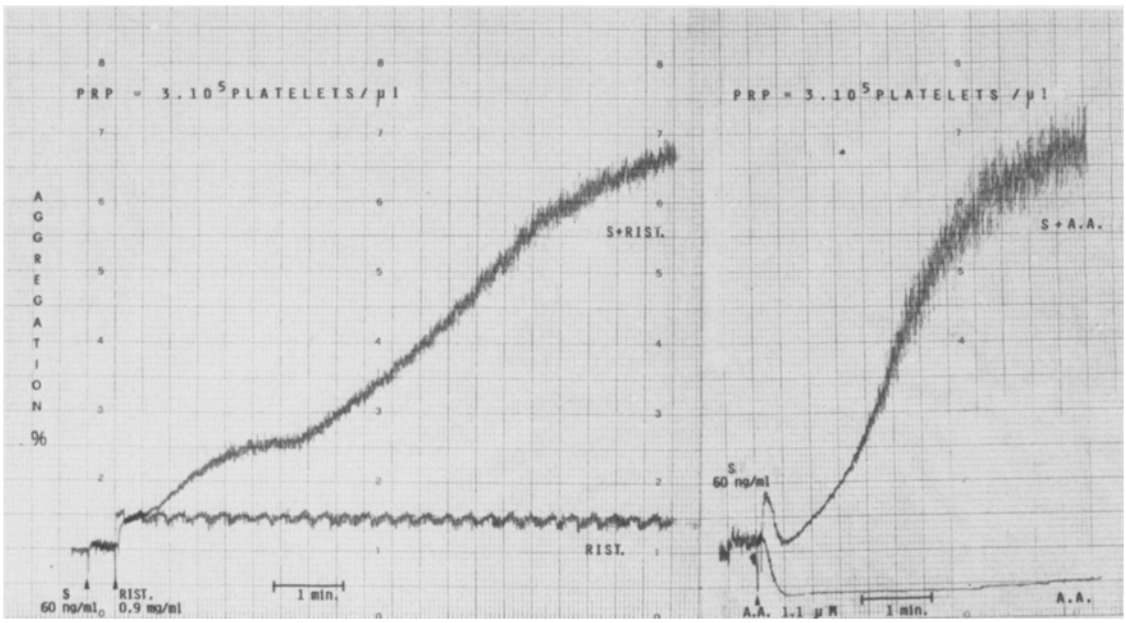

Figure 1. Somatostatin (S) strongly enhances the effect on human PRP of subthreshold concentrations of ristocetin (RIST) (on the left) and arachidonic acid (A.A.) (on the right).

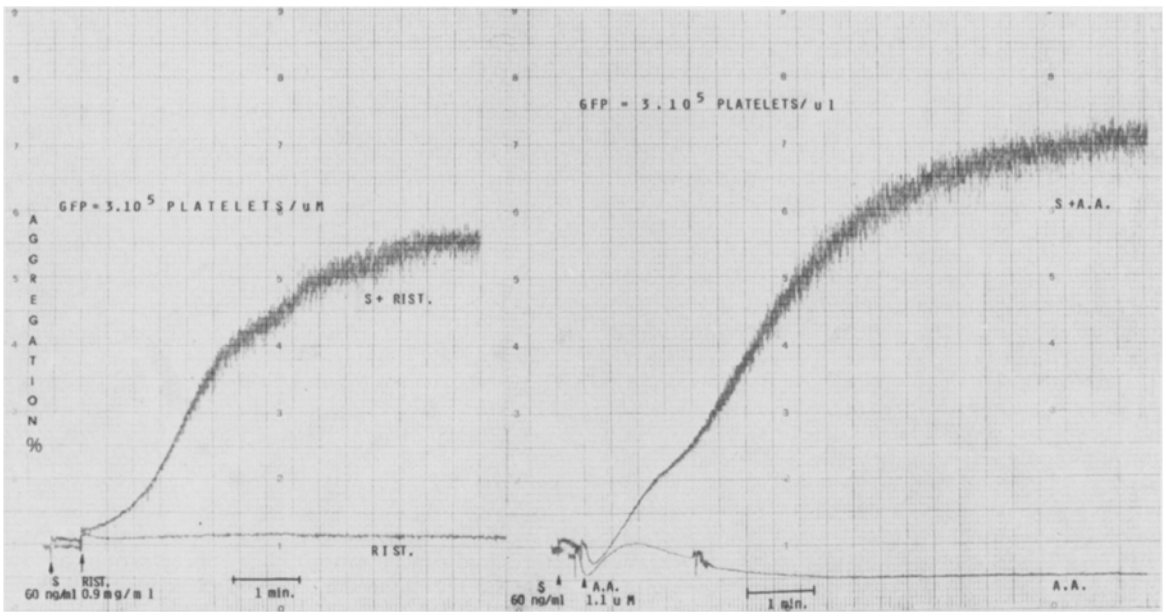

Figure 2. Somatostatin (S) strongly enhances the effect on human gel filtered platelets (GFP) of subthreshold concentrations of ristocetin (RIST) (on the left) and arachidonic acid (A.A.) (on the right).

effects at concentrations of 1 ng/ml, 300 ng/ml and 1 µg/ml; the effect of 250 pg/ml of S-14 was not statistically significant, although in some experiments concentrations of S-14 as low as 50 pg/ml were effective. In the range from 1 ng/ml to 1 µg/ml the effect of S-14 was not dose-dependent. The enhancing effect was maximal when the interval between addition of S-14 and addition of the aggregation inducer was in the range of 10 s–5 min, but markedly decreased at intervals of 5–20 min and was lacking after long-lasting contact of S-14 with PRP (more than 20 min). When, after intervals of 20 min or more, a second identical dose of S-14 was applied to specimens of PRP, previously treated with 60 ng/ml of S-14, a lack of enhancing effect was observed. No enhancing effect of S-14 was observed on the platelet aggregation induced by ADP or epinephrine (table). Gel-filtered platelet preparations were similarly stimulated by previous addition of S-14 (in the above range of concentrations) to aggregate in response to subthreshold concentrations of arachidonic

Effect of somatostatin (S-14) (60 ng/ml) on the percent of aggregation of normal PRPs stimulated with subthreshold concentrations of collagen, arachidonic acid, ristocetin, epinephrine and ADP

Collagen			Arachidonic acid			Ristocetin			Epinephrine			ADP		
%			%			%			%			%		
µg/ml	−	+ S-14	µM	−	+ S-14	mg/ml	−	+ S-14	µM	−	+ S-14	µM	−	+ S-14
0.1	0	73	0.55	0	87	0.5	2	32	1.0	3	4	0.2	8	3
0.3	27	41	0.60	4	82	0.5	5	84	1.0	3	3	0.4	6	5
0.3	10	45	0.70	0	72	0.6	3	45	1.0	7	7	0.4	15	5
0.4	0	72	0.75	3	65	0.7	5	77	1.0	4	8	0.6	4	6
0.5	5	70	0.80	0	76	0.9	0	72	5.0	32	27	0.6	25	25
0.6	0	18	0.80	6	82	0.9	0	88	5.0	10	15	0.8	7	12
1.0	36	57	1.00	5	68	1.0	0	20	5.0	10	5	0.8	3	2
2.0	17	67	1.10	0	88									
2.0	48	87												
Mean	15.9	51.9		2.2	77.5		2.1	59.7		9.8	9.8		9.4	8.28
± SD	17.6	26.6		2.5	8.6		2.3	27.8		10.2	8.5		7.1	8.36
$p < 0.001$			$p < 0.001$			$p < 0.001$			$p < 0.3$ N.S.			$p < 0.3$ N.S.		

− = control; + S-14 = after somatostatin. Statistical evaluation by means of Aspin-Welch test.

acid, ristocetin and collagen (fig. 2), but not of ADP or epinephrine. None of the reported effects were observed when only the vehicle of S-14 was used.

Our data provide some evidence of a direct effect of somatostatin, under in vitro conditions, on normal human platelets. The selectivity of the enhancing effect of somatostatin on the platelet aggregation induced by collagen and ristocetin and not on that induced by ADP or epinephrine deserves further investigation, with reference to the different mechanisms by which the agonists we used induce platelet aggregation. Ristocetin induces platelet aggregation by mediating the binding of plasma vWF to the platelet membrane glycoprotein Ib[20]; the collagen molecule contains a relatively wide distribution of platelet-binding sites, some of which consist of amino acid sequences also interacting with platelet glycoprotein Ib[21, 22]; however, platelet stimulation by both ADP and epinephrine requires a more complex interaction involving fibrinogen binding to specific recognition sites (glycoproteins IIb and IIIa). Our hypothesis is that somatostatin can modulate specific binding sites on the platelet membrane, perhaps the platelet glycoprotein Ib.

The desensitization phenomena observed when somatostatin was repeatedly applied to the same specimen of PRP also point to a mechanism of interaction at recognition sites. Specific somatostatin recognition sites on the platelet membrane have not been identified, but it is noteworthy that the somatostatin binding sites recently characterized in CNS as well as in many different types of peripheral tissues transduce a signal leading to inhibition of cAMP production, via coupling of somatostatin binding sites with the regulatory subunit of the GTP-dependent adenylate cyclase[18, 23]: a reduction of platelet cAMP levels may well account for the enhancing effect of somatostatin on the platelet aggregation. It is also noteworthy that the in vitro concentration levels of somatostatin effective in our experiments were similar to those obtained in vivo by somatostatin infusion in man, suggesting a role of the platelet/somatostatin interaction in the hemostatic effect of this peptide.

1 Kayasseh, L., Gyr, K., Stalder, G. A., and Allgöwer, M., Lancet *2* (1978) 833.
2 Coraggio, F., Scarpato, P., Spina, M., and Lombardi, S., Br. med. J. *289* (1984) 224.
3 Kayasseh, L., Gyr, K., Keller, U., Stalder, G. A., and Wall, M., Lancet *1* (1980) 844.
4 Somerville, K. W., Davies, J. G., Hawkey, C. J., Henry, D. A., Hine, K. R., and Langman, M. J. S., Lancet *1* (1985) 130.
5 Magnusson, I., Ihre, T., Johansson, C., Seligson, U., Torngren, S., and Uvnas-Moberg, K., Gut *26* (1985) 221.
6 Torres, J., Landa, I., Hernandez, F., Jove, J. M., Suarez, A., Arias, J., Cuberes, R., Santoy, J., Fernandez, R., Calleja, J., Nisa, E., Rodriguez, J. L., Moreno, E., and Balibrea, J. L., Br. J. Surg. *73* (1986) 786.
7 Basso, N., Bagarani, M., Bracci, F., Cucchiara, G., Gizzonio, D., Grassini, G., Percoco, M., Procacciante, F., and Toti, F., Arch. Surg. *121* (1986) 833.
8 Koerker, D. J., Harker, L. A., and Goodner, C. J., New Eng. J. Med. *24* (1975) 476.
9 Besser, G. M., Paxton, A. M., Johnson, S. A. N., Moody, E. J., Mortimer, C. H., Hall, R., Gomez-Pan, A., Schally, A. V., Kastin, A. J., and Coy, D. H., Lancet *1* (1975) 1166.
10 Fuse, I., Ito, S., Takagi, A., and Shibata, A., Life Sci. *36* (1985) 2047.
11 Mielke, C. H., Gerich, J. E., Lorenzi, M., Tsalikian, E., Rodvien, R., and Forsham, P. H., New Eng. J. Med. *24* (1975) 480.
12 Giugliano, D., Coppola, L., Misso, L., Tirelli, A., Passariello, N., and D'Onofrio, F., Metabolism *30* (1981) 172.
13 Di Tullio, N. W., Kinter, L. B., and Storer, B. L., Thromb. Res. *40* (1985) 425.
14 Vittet, D., Rondot, A., Cantau, B., Launay, J. M., and Chevillard, C., Biochem. J. *233* (1986) 631.
15 Siess, W., Stifel, M., Binder, H., and Weber, P. C., Biochem. J. *233* (1986) 83.
16 Filep, J., and Rosenkranz, B., Thromb. Res. *45* (1987) 7.
17 Bichet, D. G., Arthus, M. F., Barjon, J. N., Lonergan, M., and Kortas, C., J. clin. Invest. *79* (1987) 881.
18 Lewin, M. J. M., Scand. J. Gastroent. *21* (Suppl. 119) (1986) 42.
19 Tangen, O., and Berman, H. J., Adv. exp. Med. Biol. *34* (1972) 235.
20 Coller, B., Peerschke, E., Scudder, L., and Sullivan, C., Blood *61* (1983) 99.
21 La Duca, F. M., Bettigole, R. E., Bell, W. R., and Robson, E. B., Blood *68* (1986) 927.
22 Fitzsimmons, C. M., Cawston, T. E., and Barnes, M. J., Thromb. Haemost. *56* (1986) 95.
23 Axelrod, J., Discussion in Neurosciences, vol. III, n. 3, p. 132. FESN, Geneva 1986.

0014-4754/88/100892-03$1.50 + 0.20/0