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IN VITRO EFFECTS OF SOMATOSTATIN ON RED CELL FILTERABILITY MEASURED BY THREE METHODS

Action *in vitro* de la somatostatine (SRIF) sur la filtrabilité érythrocytaire mesurée par trois méthodes

J.F. BRUN, M. RAUTURIER, Y. GHANEM, A. ORSETTI

Service d'Exploration Physiologique des Hormones et des Métabolismes, Professeur A. Orsetti, Hôpital Lapeyronie, F 34079 Montpellier Cedex and Laboratoire de Physiologie 2, Institut de Biologie, Faculté de Médecine, F 34060 Montpellier Cedex

ABSTRACT :

In vitro effects of somatostatin on red cell filterability measured by three methods.

Somatostatin is an ubiqutary neuropeptide and hormone which has been reported to exert hemodynamic effects and to bind to receptors on the red cell membrane. We investigated its effects on red cell deformability by three filtration methods : (a) filtration of red cells resuspended at hematocrit 8 % in Tris-Albumin-Glucose buffer under atmospheric pressure ; (b) filtration of red cells resuspended at hematocrit on native plasma at 8 % hematocrit under a negative pressure of 5 cm of water ; (c) filtrability of whole blood under a negative pressure of 20 cm of water. Aprotinin (Antagosan*) was added to the different suspensions in order to avoid rapid destruction of somatostatin. Increased quantities of somatostatin (from 1 pg/ml to 1 µg/ml) were obtained by adding natural somatostatin (Modustatin*) to the media, before they were incubated at 37 C for 30 minutes. In 11 samples from healthy subjects, somatostatin was shown to increase red cell flow rate in technique (c) (+ 118 %, $p<0.05$) and to reduce red cell rigidity index in technique (b) (− 71 %, $p<0.025$) whereas a nonsignificant similar tendency (− 56 %) was observed with technique (a). Similar results ($p<0.05$) are observed when adding somatostatin to blood of diabetics. These *in vitro* data suggest that somatostatin, like other previously studied hormones, may modify red cell deformability.

Key-words : Somatostatin. Erythrocyte deformability. Hemorheology. Diabetes.

RÉSUMÉ :

Action _in vitro_ de la somatostatine (SRIF) sur la filtrabilité érythrocytaire mesurée par trois méthodes.

La somatostatine est un neuropeptide et une hormone ubiquitaire, auquel on a attribué des actions hémodynamiques et qui se fixerait sur des récepteurs érythrocytaires. Nous avons étudié ses effets sur la déformabilité érythrocytaire avec trois méthodes de filtration : (a) filtration de globules rouges resuspendus à hématocrite 8 % dans un tampon Tris-Albumin-Glucose sous pression atmosphérique ; (b) filtration de globules rouges resuspendus à hématocrite 8 % dans leur plasma d'origine sous pression négative de 20 cm d'eau. L'aprotinine (Antagosan*) était ajoutée pour éviter la destruction rapide de la somatostatine. Des concentrations croissantes de somatostatine (de 1 pg/ml à 1 µg/ml) ont été réalisées par ajout de Modustatine*, avant incubation de 30 min à 37 C. Sur 11 échantillons de sujets sains, la somatostatine accroît le débit de filtration érythrocytaire dans la technique (c) (+ 118 %, $p < 0.05$) et réduit l'index de rigidité dans la technique (b) (− 71 %, $P < 0.025$) avec une tendance non significative dans le même sens (− 56 %) pour la technique (a). Des résultats similaires ($p < 0.05$) sont observés en ajoutant somatostatine à du sang de diabétiques. Ces résultats préliminaires *in vitro* suggèrent l'existence d'une action de somatostatine, comme d'autres hormones déjà étudiées, sur la déformabilité des érythrocytes.

Mots-clés : Somatostatine. Déformabilité érythrocytaire. Hémorhéologie. Diabète.

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Tirés à part : Dr J.-F. BRUN, Laboratoire de Physiologie 2, Institut de Biologie, Faculté de Médecine, F 34060 Montpellier Cedex.

Somatostatin, a tetradecapeptide originally isolated from the hypothalamus, has been shown to be a widely distributed tissue component, that in some settings acts as a paracrine secretion and in others as a neuroendocrine secretion (4). Studies in dogs have demonstrated that 16 000 MW somatostatinlike immunoreactivity is also released into the circulation from the stomach and pancreas after the ingestion of a meal (9). In man, the hormonal status of soma-

tostatin has been more difficult to prove, largely because of technical problems involving its measurement in human plasma. Zyznar and coworkers (9) gave the first demonstration in man that 1 600-dalton somatostatinlike immunoreactivity (*i.e.* the low molecular, biologically active moity) rises in both normal and diabetic humans after a mixed meal. Therefore, somatostatin is a circulating hormone (7) and is found in plasma at concentrations (between 8 and 25 pg/ml) which have been shown to exert physiological effects.

In addition, some circulatory effects of somatostatin have been described. Somatostatin decreases mesenteric blood flow, increases mesenteric vascular resistance (4), increases peripheral blood flow (6), inhibits platelet function (3) as well as release of mediators from human basophils.

Since somatostatin, like other regulatory peptides, is known to bind to specific receptors on the red cell membrane, we investigated a possible direct action of this hormone on red cell deformability.

MATERIALS AND METHODS

Blood samples

The study was performed by *in vitro* infusion of whole blood and erythrocyte suspensions at 37° C. Human blood was obtained by venipuncture (in the cubital fossa) from 11 healthy female volunteers (age : 23-38 yr) and 6 diabetic women (age : 26-52 yr). All blood samples were drawn in the laboatory for immediate processing. They were anticoagulated with potassium EDTA (0.38 mol/l). Diabetic women were all treated by portable intraperitoneal insulin delivery systems, with glycosylated hemoglobin values (HbA1) ranging between 7 and 9 %.

In vitro incubations

Somatostatin (Modustatine, Clin Midy Pharmaceuticals, Montpellier, France) was solubilized in tris-albumin-glucose (TRAG) buffer at increasing concentrations so that 100 µl of this solution could be added to blood or red cell suspensions. The TRAG buffer was made with the following reagents (for 1 000 ml) : NaCl 8.5 g ; KCl 0.3 g ; $CaCl_2$ $2H_2O$ 0.3 g ; Glucose : 1 g ; Serum albumin : 0.5 g ; Tris M 10 ml. The pH was then adjusted at 7.4. Control values (red cell preparations with no addition of somatostatin) were measured in samples in which TRAG buffer alone was added. In the first series of experiments (*i.e.* in control subjects), we added aprotinin (Antagosan, Hoechst pharmaceuticals) to avoid rapid proteolysis of somatostatin by plasma enzymes. However, in the experiements on diabetics, we did no longer add this reagent.

Hemorheologic measurements

Blood and erythrocyte filterability were measured on 5 µm Nuclepore sieves (kindly offered by Hoechst Pharamaceuticals). Sieves were reused after ultrasonic cleaning as previously reported (2) so that the coefficient of variation for measurements performed with the same sieve is about 3 %. All sieves were from the batch No 54 P4 B5.

Whole blood filterability was measured according to Reid (5), and expressed as a flow rate of red cells passing through the sieve under 200 mm negative pressure.

Red cell filterability was determined by two methods. Firstly, by measuring the time of passage of 1 ml of resuspended washed red cells in TRAG buffer (hematocrit 8 %) under atmospheric pressure with the technique described by Weill and coworkers (8). Then, by measuring the time of passage of washed red cells resuspended on native plasma at 8 % hematocrit, with a driving pressure of 50 mm of water. Hanss's index of red cell rigidity (IFH) as calculated with the following formula :

$$IFH = \frac{Te - Tb}{Tb} \times \frac{100}{h}$$

in which Tb is the time of passage of suspending medium, Te the time of filtration of resuspended erythrocytes and h the hematocrit of the suspension.

All hematocrits (packed cell volume) were measured by microcentrifugation.

Statistics

Values are given as mean ± SEM. Statistical comparisons were performed by nonparametric tests : Wilcoxon rank sum test for paired data and Mann-Whitney test for unpaired data. Previous calculation of overall statistical comparisons was performed by analysis of variance. Correlations (linear regressions) were calculated by least square fitting. Statistical significance was defined as $p<0.05$).

RESULTS

Incubations of red cells from healthy subjects

Figure 1 shows that somatostatin, at the concentrations of 1 and 10 pg/ml, significantly increases whole blood filterability. When somatostatin quantities increase above this physiological range, this effect is no longer observed.

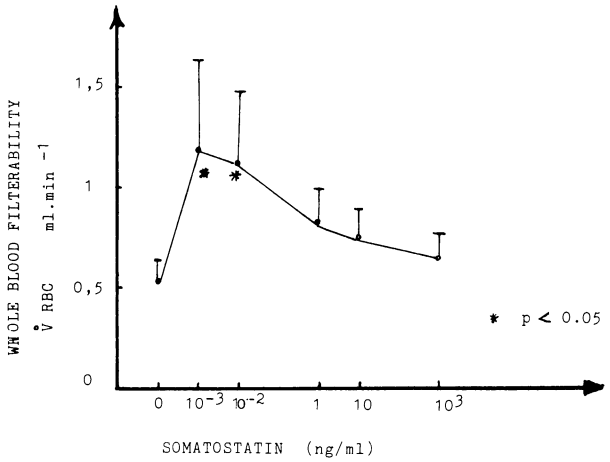

FIG. 1. — *Effects of* in vitro *addition of somatostatin on whole blood filterability. Driving pressure : 200 mm of water. Values : mean ± SEM. Red cell flow rate is increased.*

Effects de l'addition in vitro de somatostatine sur la filtrabilité sanguine totale. Pression : 200 mm d'eau. Valeurs : moyenne ± SEM. Le débit de filtrabilité des érythrocytes est accru.

Figure 2 shows that somatostatin, at all concentrations, reduces the rigidity index of red cells ($p < 0.025$, analysis of variance). A nonsignificant similar tendency is found in *figure 3* with the filtration of red cells resuspended on native plasma.

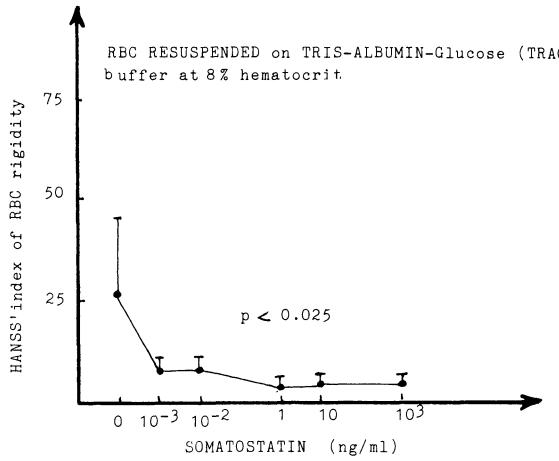

FIG. 2. — *Effects of* in vitro *addition of somatostatin on erythrocyte rigidity index during measurements of filterability of resuspended red cells on TRAG buffer at hematocrit 8 %, under atmospheric pressure. Values : mean ± SEM Red cell rigidity index is decreased.*

Effets de l'addition in vitro de somatostatine sur la filtrabilité d'érythrocytes lavés resuspendus à 8 % d'hématocrite dans un tampon TRAG, sous pression atmosphérique. Valeurs : moyenne ± SEM. L'index de rigidité des érythrocytes est diminué.

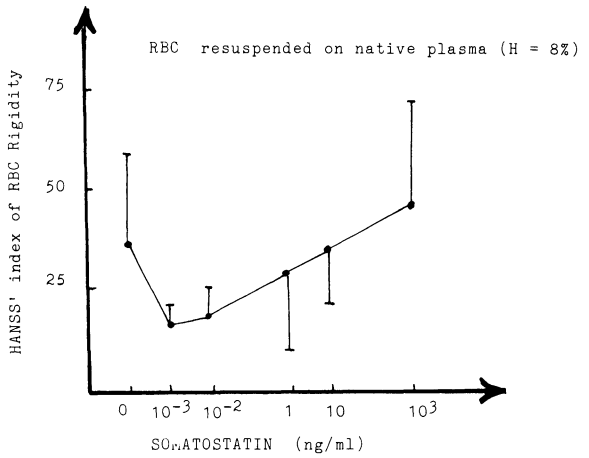

FIG. 3. — *Effects of* in vitro *addition of somatostatin on erythrocyte rigidity index during measurements of filterability of resuspended red cells on native plasma at hematocrit 8 %. Driving negative pressure : 50 mm water. Values : mean ± SEM nonsignificant tendency to a reduction of red cell rigidity index.*

Effect de l'addition in vitro de somatostatine sur la filtrabilité d'hématies resuspendues à hématocrite 8 % dans leur plasma d'origine. Pression : 50 mm d'eau. Valeurs : moyenne ± SEM. Il existe une tendance non significative à la réduction de l'index de rigidité des érythrocytes.

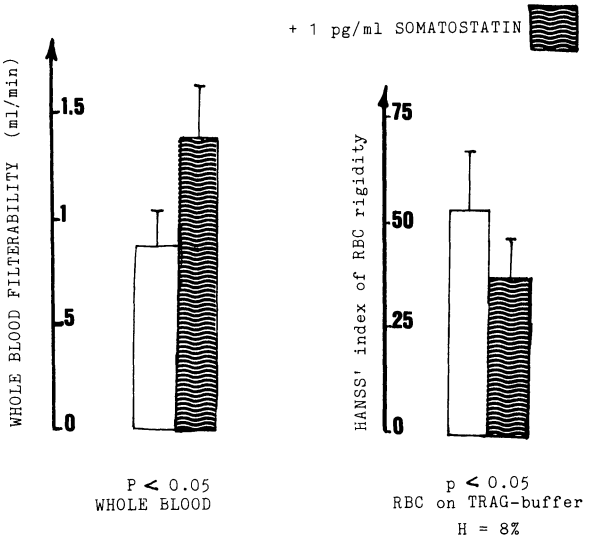

FIG. 4. — *Effects of* in vitro *addition of somatostatin on erythrocytes from diabetic subjects. Right : rigidity index during filtration of resuspended red cells on TRAG buffer at hematocrit 8 %. Left : red cell flow rate measured during whole blood filtration tests. Values : mean ± SEM. Erythrocite rigidity is reduced and red cell flow rate is increased.*

Effects de l'addition in vitro de somatostatine sur les érythrocytes de sujets diabétiques. A droite : index de rigidité des globules rouges lors de la filtration de suspension d'hématies resuspendues à hématocrite 8 % dans un tampon TRAG. A gauche : débit de filtrabilité érythrocytaire mesuré lors de tests de filtrabilité sanguine totale (pression : 200 mm d'eau). Valeurs : moyenne ± SEM. L'index de rigidité érythrocytaire est diminué et le débit de filtrabilité des érythrocytes sur sang total est accru.

INCUBATIONS OF RED CELLS FROM DIABETIC WOMEN

The preliminary results of the study of the effects of somatostatin on red cells from diabetics is shown on *figure 4*. Only the two methods which gave significant results in the experiments on normal subjects were used. It is shown that addition of 1 pg/ml of somatostatin *in vitro* increases red cell filterability (measured on whole blood) and reduces erythrocyte rigidity index (measured on TRAG buffer). Both results are significant at $p < 0.05$.

DISCUSSION

The results of the present experiments strongly suggest that somatostatin, when directly added *in vitro* to red cell suspensions, increases erythrocyte deformability in both normal and diabetic subjects. It must be emphasized that the data are preliminary and further testing of somatostatin is required. Nevertheless, a number of tentative points may be addressed.

If one considers the values reported *in vivo* by Zyznar and coworkers (9) the *in vitro* effects of somatostatin found in our study occur at physiological concentrations (1 and 10 pg/ml). This could suggest that circulating levels of somatostatin which are achieved in peripheral blood under physiological conditions may result in an increase of red cell deformability.

Since somatostatin generally acts as an inhibitor of many functions in the body (1), we were expecting, if any, rather an inhibitory effect on red cell rheology. However, this increase of red cell deformability which is suggested by our finding may explain the recently reported effect of somatostatin on peripheral blood flow, which seems to be stimulatory (6).

During glucose clamp studies in nondiabetic subjects, we observed an increase in red cell filterability, which could be the *in vivo* consequence of the effects we report here (J.F. Brun, unpublished data). However, it will probably be difficult to confirm *in vivo* the rheologic actions of somatostatin, since this hormone acts on many different targets and exerts simultaneously a great number of effects.

Further studies will be required to confirm our findings. However, our data suggest that somatostatin, like other hormones previously studied, modifies red cell deformability. Interestingly, in contrast with most other hormones and chemical messengers (epinephrine, glucagon, leukotriene C4, leukotriene B4), somatostatin does not increase red cell rigidity and seems to reduce it.

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