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Somatotropin and somatostatin effects on vitamin K-dependent plasma coagulation factors

Negrin Negrev, Yuri Nyagolov *, Emiliya Stanchewa

Department of Physiology, Medical University, 9002 Varna, Bulgaria

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Abstract

The effects of somatotropin (0.2 mg/kg body mass) and somatostatin (0.1 mg/kg body mass) on plasma coagulation factors II, VII, IX, X and some general indexes of hemocoagulation were examined. Hormones were injected subcutaneously in male Wistar rats on 3 consecutive days. Boehringer Mannheim tests and Schnitger and Gross coagulometer were used for clotting factor determination. Somatotropin caused significantly decreased activity of factors II, VII and X ($P < 0{,}001$) and IX ($P < 0.05$). Somatostatin alone, as well as somatotropin after somatostatin pretreatment considerably increased the activity of factors II, VII and X ($P < 0{,}001$), while factor IX was non-significantly suppressed. It is concluded that somatotropin and somatostatin are possible regulators of biosynthesis of vitamin K-dependent plasma coagulation factors. Somatotropin depresses the activity of factors II, VII, IX and X and causes hypocoagulability, while somatostatin not only prevents the inhibiting effect on factors II, VII and X, but also increases their activity and causes hypercoagulability.

Keywords: Somatotropin; Somatostatin; Hemocoagulation; Vitamin K-dependent coagulation factor

1. Introduction

Participation of the endocrine system in hemocoagulation is indisputable (Colwell, 1993; Klein et al., 1993; Stubblefield, 1993; Tschoepe et al., 1993), but its specific role is insufficiently elucidated. Somatotropin and somatostatin have attracted attention, because of the scarcity of information about their involvement in hemocoagulation, and because of the extensive evidence of their capability to influence a large number of processes in the liver. The liver is postulated to be the basic source of plasma hemocoagulation factors (Davie, 1987), and also the site of synthesis and γ-glutamyl carboxylation of vitamin K-dependent plasma coagulation and anticoagulation proteins (Bovill et al., 1993). Data show that somatostatin not only exerts its effects as a regulator of the episodic secretion of somatotropin (Robinson et al., 1990) and as a gastrointestinal hormone (Klaff et al., 1988), but it is present in hepatic nerves as a neurotransmitter as well (Feher et al., 1992). Moreover somatotropin and somatostatin receptors (binding sites) are expressed in hepatocytes (Robinson et al., 1993; Raper et al., 1992), while investigators have provided convincing evidence that these hormones are important regulators of regeneration and growth of liver cells (Husman and Andersson, 1993; Hashimoto et al., 1993), and of liver metabolism (Davidson, 1987; Rosa et al., 1992). On this basis this study was aimed to investigate the effects of somatotropin and somatostatin on vitamin K-dependent plasma coagulation factors II, VII, IX and X in rats.

2. Materials and methods

2.1. Animals

Experiments were carried out on 86 white male Wistar rats weighing 200–220 g, housed under optimal conditions, with free access to standard food and water. Some of the animals (56) were used in the experiment, while the rest were a source of standard plasma for prothrombin index determination and construction of calibration curves.

* Corresponding author. Department of Physiology, Medical University, Marin Drinov Street 55, 9002 Varna, Bulgaria

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2.2. Design of hormone administration

The test animals were divided into four equal groups. Hormones were administered subcutaneously once daily for 3 consecutive days. The first group of rats was injected with somatotropin (Somatotropin, human, Boehringer Mannheim, activity 1.8 units/ml) 0.2 mg/kg body mass, the second group – with somatostatin (Tyr-somatostatin, Sigma, minimum 97%) 0.1 mg/kg body mass, the third group – with the two hormones, using the same procedure and doses, as somatostatin was applied 3 h before somatotropin. The doses of the hormones were determined experimentally by examination of logarithmic dose-effect dependence and according to literature data (Bak, 1993; Robinson et al., 1990). The fourth group was injected with saline (the solvent of the hormones), using the same scheme and volume per kg body mass.

2.3. Collection of blood and procedure for plasma acquisition

The necessary blood volume from one rat was taken into plastic syringes by cardiac puncture under ether narcosis. Sodium citrate (0.11 mol) was used as an anticoagulant. Citrated blood was centrifuged for 10 min at 2000 × g. The citrated plasma was stored at 4° C and the parameters to be examined were determined not later than after 2 h.

2.4. Determination of indexes of hemocoagulation

Bioactivities of plasma clotting factors II, VII, IX and X were estimated by means of Boehringer Mannheim coagulation tests and methods used according to the instructions. Clotting times were measured with a Schnitger and Gross coagulometer and the activity of the plasma factor examined was determined from calibration curves in units of activity; 100% activity corresponded to 1 unit/ml according to Molho-Sabatier et al., (1992). The overall indexes of hemocoagulation, activated partial thromboplastin time and prothrombin time expressed as prothrombin index were determined by routine methods (Lisichkov, 1987), using tests of the National Institute of Haematology and Transfusiology – Sofia, and the coagulometer of Schnitger and Gross.

2.5. Construction of calibration curves

Standard plasma from 30 healthy male Wistar rats which were not treated with anything was obtained according to the directions of the Boehringer Mannheim tests, was serially diluted and after measurement of clotting times, calibration curves were plotted on double logarithmic paper for each of the plasma factors.

2.6. Statistical analysis

The data were analysed by variation analysis, applying Student-Fisher's t-test.

3. Results

3.1. Influence of somatotropin and somatostatin on plasma coagulation factors II, VII, IX and X

The results are presented in Fig. 1. Somatotropin applied alone lowered the activity of factor II by 64.23% ($P < 0.001$), factor VII by 67.29% ($P < 0.001$), factor IX by 11.86% ($P < 0.05$), and factor X by 66.13% ($P < 0.001$). Separate somatostatin administration increased the activity of factor II by 68.96% ($P < 0.001$), factor VII by 71.01% ($P < 0.001$) and factor X by 64.87% ($P < 0.001$), while that of factor IX was non-significantly decreased. Somatotropin after somatostatin pre treatment increased the activity of factor II by 69.16% ($P < 0.001$), factor VII by 66.54% ($P < 0.001$), and factor X by 60.48% ($P < 0.001$), while the activity of factor IX was decreased by 11.16%.

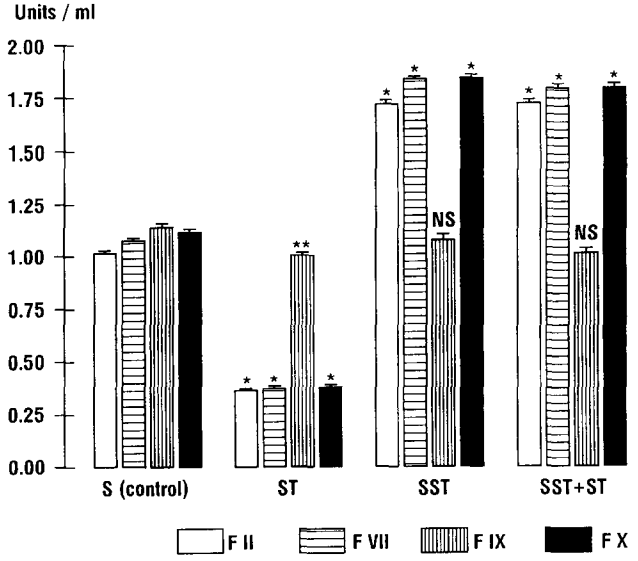

Fig. 1. Effects of somatotropin (0.2 mg/kg body mass) and somatostatin (0.1 mg/kg body mass) on vitamin K-dependent plasma hemocoagulation factors II, VII, IX and X in male Wistar rats after 3 days' s.c. treatment. Data are presented as means ± S.E.M. Ordinate – activity of plasma factors expressed in units/ml (100% activity = 1 unit/ml). The following abbreviations and symbols are used: ST – somatotropin; SST– somatostatin; SST + ST – somatostatin administered three hours before somatotropin; S–saline; * $P < 0.001$, * * $P < 0.05$; N.S. – non-significant.

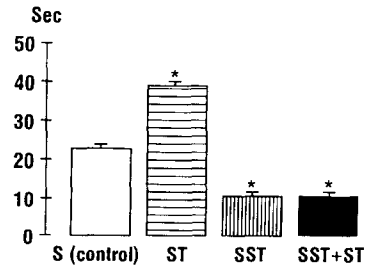

Fig. 2. Effects of somatotropin and somatostatin on activated partial thromboplastin time. Data are presented as means ± S.E.M. Ordinate – activated partial thromboplastin time in s. Abbreviations and symbols are as in Fig. 1.

3.2. Influence of somatotropin and somatostatin on activated partial thromboplastin time and prothrombin index

Changes in activated partial thromboplastin time are presented in Fig. 2. Somatotropin increased this parameter by 70.61% ($P < 0.001$), while somatostatin shortened it by 53.44% ($P < 0.001$). Somatotropin after somatostatin pretreatment shortened the activated partial thromboplastin time by 52.87% ($P < 0.001$). Similar shifts were observed for the prothrombin index (Fig. 3), which decreased by 56.44% ($P < 0.001$) after somatotropin treatment, while somatostatin alone, and somatostatin pretreatment with subsequent somatotropin administration increased it respectively by 64.21% ($P < 0.001$) and 68.34% ($P < 0.001$).

4. Discussion

The data are evidence that somatotropin (Fig. 1) significantly decreases plasma hemocoagulation factors II, VII, IX and X activities. This could be accepted as a probable sign of inhibited biosynthesis of these factors. It is hard to define the mechanism of the action of the hormone, but it is quite possible that its suppressing effect can be exerted in the liver. This is supported by the fact that the plasma coagulation factors II, VII, IX and X are mainly synthesised in the liver (Davie, 1987). A significant part of the various effects of somatotropin is exerted in the liver (Daughaday et al., 1972; Davidson, 1987). Moreover, at least an indirect effect of somatotropin cannot be excluded. In support of this, somatotropin binding to lactogenic receptors (Roguin et al., 1990) or auto-regulation of its own receptors (Barash and Posner, 1989; Robinson et al., 1993) and secretion (Lea and Harvey, 1992) could be considered.

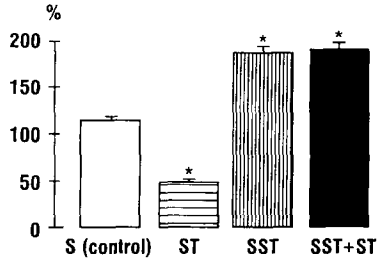

Fig. 3. Effects of somatotropin and somatostatin on prothrombin time expressed as prothrombin index. Data are presented as means ± S.E.M. Ordinate – prothrombin index in %. Abbreviations and symbols are as in Fig. 1.

Separate administration of somatostatin (Fig. 1) is followed by a significant increase in the activity of factors II, VII and X. It could be hypothesised that somatostatin activates their generation. The effect of this hormone is probably connected with its capability to inhibit synthesis and secretion of endogenous somatotropin (Robinson et al., 1990), and to reduce its plasma activity and effects. This phenomenon could be interpreted as removing the depressing effect of endogenous somatotropin on biosynthesis of the factors in question, as far as the above data about exogenous somatotropin are consequent with this possibility. This might be an explanation for activities of factors II, VII and X elevated by somatostatin.

Literature data demonstrating that liver is a target organ of somatostatin (Raper et al., 1992), might be taken into consideration as an alternative explanation for the stimulating effect of this hormone on plasma coagulation factors II, VII and X. Moreover, somatostatin is reported to exert a hepatic cytoprotective effect (Landa et al., 1992).

Analysis of results reflecting the influence of somatotropin after somatostatin pretreatment is of particular importance (Fig. 1). First, it is clear that the activities of plasma factors II, VII and X are significantly increased, while factor IX is non-significantly depressed. On one hand this could be evidence that somatostatin pretreatment effectively prevents the inhibiting effect of somatotropin. On the other hand, somatostatin pretreatment completely reproduces the results of the experimental design with separate administration of the hormone, even with regard to factor IX. It is obvious that these facts do not contradict the finding that somatostatin causes central effects (Robinson et al., 1990), as well as peripheral effects (Raper et al., 1992), but they demonstrate that by mechanism(s) presently unknown, at least one of these effects is connected with hemocoagulation by causing increased activation of II, VII and X plasma factors.

Striking is the observation that factors II, VII and X reacted in a comparable way, in contrast to factor IX which had been shown to be less sensitive to somatotropin and non-significantly influenced by the combination of the hormones (Fig. 1). Whether this difference is a result of different effects on liver biosynthesis of the factors or is a consequence of different half-lives of these factors remains unclear.

The effects observed may concern protein synthesis or may be connected only with vitamin K-dependent γ-glutamyl carboxylation of the factors. Our unpublished data demonstrating similar effects of the same hormones on other coagulation factors, the bioactivity of which is liver-independent or which are vitamin K-independent, are in favour of the suggestion of a possible influence of the hormones on liver biosynthesis of the clotting factors investigated. This hypothesis, of course demands additional investigation.

The shifts in the values for criteria of hemocoagulation, the activated partial thromboplastin time (Fig. 2), and prothrombin index (Fig. 3) demonstrate the significant hypocoagulability caused by somatotropin. This phenomenon might be normally connected with the depressed activity of plasma coagulation factors II, VII, IX and X already depicted (Fig. 1).

Somatostatin administered alone, as well as somatostatin followed by somatotropin, shortened the activated partial thromboplastin time (Fig. 2), and increased the prothrombin index (Fig. 3). This is evidence, that in this case, somatostatin causes hypercoagulability, which most probably is an expression of, or at least is connected with, significantly activated bioactivity of plasma coagulation factors II, VII and X (Fig. 1). The fact that the activated partial thromboplastin time reflects the processes of coagulation via the intrinsic pathway, and the prothrombin index – coagulation via the extrinsic pathway, should not be ignored (McGee and Li, 1991). The changes in these criteria suggest that the hypocoagulability caused by somatotropin and the hypercoagulability caused by somatostatin are clearly connected with plasma factors II, VII, IX and X. Nevertheless, an effect of these hormones on other coagulation factors, as well as on anticoagulant and/or fibrinolytic systems cannot be excluded. The finding that somatostatin increases factors II, VII and X and causes hypercoagulability, throws some light on the unexplained clinical observations of Jaramillo et al. (1991), who found hypercoagulability in patients with oesophageal bleeding varices treated with somatostatin.

Because of the widespread use of somatotropin in the treatment of short stature children (Ritzen et al., 1993) and of somatostatin and its synthetic analogue, octreotide, in the current treatment of acromegaly and gastroenteropancreatic endocrine tumours (Wass, 1994), the results of this study raise questions of serious clinical relevance. With respect to the doses applied in this investigation, it is worth noting that they were comparable to human doses (Darendeliler et al., 1990; Wass, 1994). Clinical practice with these hormones has shown a number of side effects concerning immune functions, metabolism etc. but no hemocoagulation disorders are reported (Ritzen et al., 1993; Wass, 1994). In this connection, the present study, though experimental, should attract the attention of physicians to possible deviations of hemocoagulation during the therapeutic use of somatotropin and somatostatin.

In summary, the data presented demonstrate that somatotropin applied alone decreases significantly the activity of vitamin K-dependent factors II, VII, IX and X, and causes hypocoagulability. Somatostatin alone increases the activity of factors II, VII and X and causes hypercoagulability. Somatostatin pretreatment followed by somatotropin administration not only mimics effects of separate somatostatin application, but completely removes the suppressing effects of exogenous somatotropin on the plasma factors under study and on overall hemocoagulation parameters. The data do not allow us to establish precisely the site and mechanism(s) of the action of the hormones. Most probably they influence the biosynthesis of vitamin K-dependent plasma coagulation factors in the liver.

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