Determination of certain physical and chemical characteristics of the activating serum factor from preparturient women and women with habitual abortions

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Summary. Follow-up investigation of the blood sera from preparturient women and women with habitual abortions showed the presence of a factor which has an activating effect on smooth muscle preparations because it causes the release of prostaglandins. Gel-chromatographic counter flow separation and microelectrophoresis of the blood sera have shown that the isolated serum factor is a water soluble glycopeptide with a molecular weight of about 2000.
Key words. Habitual abortions; parturition; blood serum; PG release; gel chromatography.

In earlier publications[4,5,9] we reported the presence in the blood sera of preparturient women and women with habitual abortions of a previously unidentified factor activating the spontaneous contractile activity of smooth muscles which we conditionally called the 'X' factor. Under the impact of this factor, smooth muscles release prostaglandins (PG), primarily of the $F_{2\alpha}$ type[2], through which its activating action is realized[8]. On the basis of heat-resistance data for this phenomenon was assumed that it is due to a low molecular weight polypeptide[4]. The objective of the present research was to isolate the active component from the blood serum and to determine some of its physical and chemical characteristics, using the physiological model of smooth muscle contraction for determining the biological activity.

Materials and methods. We investigated blood serum from 18 women with habitual abortions and 5 preparturient women. The method of consecutive gel filtration was applied for separation of the serum components by their mol.wt. Chromatographic columns 10/5 with Sephadex G-15, G-25 and G-200 were used. The fractions obtained, after eluting with distilled water, were tested for biological activity by registering the contractile activity of smooth muscle preparations in vitro using previously described techniques[5,9]. At the next stage of purification we studied the impact of liposolvents. The active fraction was emulgated by water-saturated butanol, the latter being extracted afterwards by fivefold treatment with ether. We applied two methods for the concentration of the biologically active fraction ultimately obtained; diaflo filtration using a diaflo cell K-50 with an UM-2 filter, and a jet of hot air at 50 °C.

Using original plates for microelectrophoresis from the Chemetron Company of Italy we obtained glyco-, lipo- and proteinogrammes which were processed by a density meter (manufactured by the firm Karl Zeiss of Jena)[6].

Results. Figure 1 presents the action of blood sera from women with habitual abortions and preparturient women on the spontaneous contractile activity of smooth muscle preparations from rat uterus under normal conditions and after administration of 10^{-6} g/ml indomethacin. It is clear from the recording that in both cases the activating effect of the blood serum is blocked completely when applied after administration of an inhibitor (indomethacin) of PG-synthetase[7].

The table presents the numbers of the fractions and the corresponding approximate mol.wt obtained by gel chromatography of blood serum from women with initiated parturition. Testing for biological activity of these fractions revealed a change in the period and amplitude of the phasic contractions of a rat uterus preparation only under the impact of fractions 4 and 7 with mol.wt approximately 1100 and 2000, respectively. The impact of fractions 7ˣ and 7′, obtained by the above method, from women with habitual abortions and preparturient women, respectively, is presented on figure 2. In both cases, the activating effect is blocked when the fractions are applied after administration of indomethacin.

The activating effect of fraction 4 from blood serum from pre-

Physiological activity of the separate fractions obtained by gel chromatography on the spontaneous contractile activity. T_1/T_2 is the ratio of the periods of the phasic contractions of smooth muscle preparations before and after treatment with the serum.

Fraction No.	mol. wt	T_1/T_2	Fraction No.	mol. wt	T_1/T_2
1	< 350	2	7	2000	1, 3
2	350	1	8	2500	1
3	700	< 1	0″	≥ 2500	
4	1100	2, 3	9	5000	1
0′	≥ 1500		10	10000	1
5	1500	1	11	15000	1
6	1750	1	12	20000	1

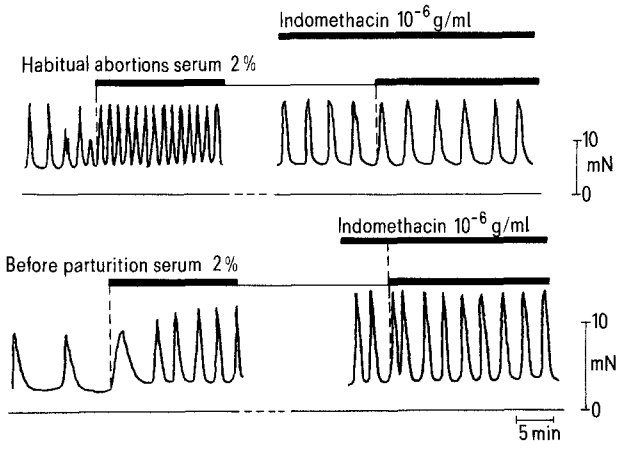

Figure 1. Presence and absence (after administration of an inhibitor of PG-synthetase) of an activating effect on rat uterus smooth muscle preparations on introduction of blood sera from preparturient women and women with habitual abortions.

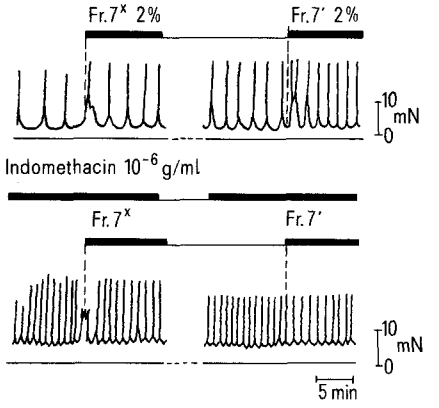

Figure 2. Results of the application of fraction 7 of blood sera from women with habitual abortions (7ˣ) and preparturient women (7′).

parturient women, containing substances of mol.wt about 1100, may be accounted for by the increased level of oxytocin (having mol.wt 1006). Therefore, blood serum from women with habitual abortions was separated into two parts and one of them was artificially contaminated with oxytocin. After separate gel filtration of the two sera, we obtained two 4^x and 7^x fractions, and we marked those of the contaminated part of the serum with asterisks. Biological testing for their effect on muscle contraction of rat uterus preparations showed (fig. 3) that only fraction 4^{x*}, i.e. the oxytocin-contaminated one, had a pronounced activating effect which meant that oxytocin was eluted in fraction 4. Both fractions 7^x and 7^{x*} retain their activating effect without significant changes which means that no oxytocin is present in fraction 7.

When studying the impact of iposolvents on fraction 7^x from blood serum from women with habitual abortions we obtained six new fractions: 7^x-1, 7^x-2, 7^x-3, 7^x-4, and 7^x-6 containing substances soluble in fatty solvents, and fraction 7^x-5 containing the water-soluble ingredients of fraction 7^x. It is clear from the results shown in figure 4 that only fraction 7^x-5 has an activating effect on smooth muscle contractile activity, and this effect is blocked when the fraction is applied after administration of Indomethacin (fig. 5), i.e. we have obtained a result identical with that given by fraction 7^x of the blood serum itself.

Microelectrophoresis revealed that fraction 7^x-5 contained no lipids. Glyco- and proteinogrammes, in density measurements, display peaks at the same point (fig. 6).

Discussion. The results, shown in figure 1, allow us to conclude that the activating effect of blood sera from women with habitual abortions and women before parturition with a nomal course of pregnancy is of the same character. In both cases, the activating effect is blocked if there has been preliminary treatment with indomethacin.

Gel filtration reveals that the blood serum from preparturient women contains only two biologically active fractions having an activating effect on smooth muscle contractile activity. Experiments with artificial contamination of serum with oxytocin (fig. 3) give us grounds to contend that the activating effect of fraction 4 of the serum is due to endogenous oxytocin.

Fraction 7, obtained by gel filtration, has an activating effect on smooth muscle contractile activity identical with that of the blood serum itself, no matter whether it is taken from women with habitual abortions or preparturient women with a normal course of pregnancy[1,9]. This allows us to claim that the activating effect of blood sera from women with habitual abortions and preparturient women is due to one and the same agent. If two different agents are responsible, they would have to have the same molecular weight and the same mechanism of action.

Experiments with liposolvents reveal that only fraction 7^x-5, containing the water soluble components of fraction 7^x, has an activating effect similar to that of the blood serum and that of fraction 7. This is a proof that we are dealing with a water soluble substance whose action is not influenced to any considerable extent by the butanol and ether with which the serum raction had been treated.

Results from microelectrophoresis show that proteino- and glycogrammes have peaks at the same point whereas a lipogramme of the same fraction shows that no lipids are present. All these results allow us to conclude that the activating serum factor from women with habitual abortions and preparturient women is the same biologically active substance in both cases, and it is a water soluble glycoprotein with a mol.wt of about 2000. Under its impact, smooth muscle cells release prostaglandins primarily of the $F_{2\alpha}$ type, which accounts for the activating effect of the serum.

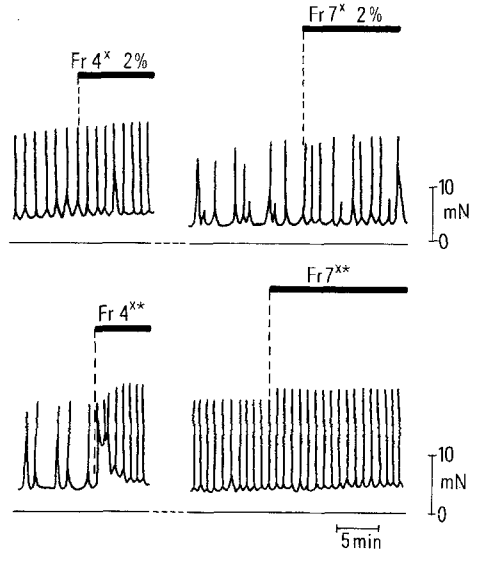

Figure 3. Changes in the spontaneous contractile activity under the impact of the different fractions of blood serum from women with habitual abortions. 4^x and 7^x are the 4th and 7th fraction of the serum obtained by gel chromatography whereas 4^{x*} and 7^{x*} are the same fractions of the same serum but contaminated with oxytocin before gel filtration.

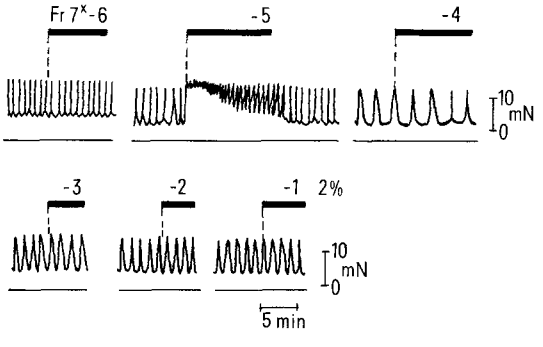

Figure 4. Among the components of fraction 7^x obtained by Craig's method, only fraction 7^x-5, containing water soluble ingredients, has an activating effect.

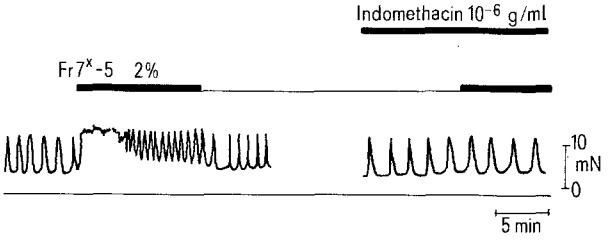

Figure 5. The activating effect of 7^x-5, the water soluble component of fraction 7^x, is inhibited after administration of a blocker of PG-synthetase.

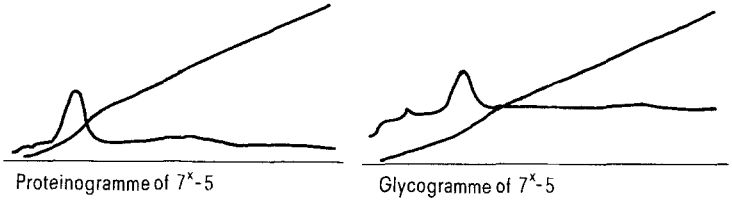

Figure 6. Glyco- and proteinogrammes of fraction 7^x-5 after density measurements.

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Bicuculline and picrotoxin block γ-aminobutryic acid-gated Cl^- conductance by different mechanisms[1]

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Summary. Using isolated, internally perfused bullfrog dorsal root ganglion cells we have studied the dose-response curves for γ-aminobutyric acid (GABA) in the presence of internally or externally applied GABA antagonists. With external application of antagonists the inhibition of the GABA current by bicuculline was competitive and that by picrotoxin was noncompetitive. Picrotoxin but not bicuculline blocked when internally perfused.
Key words. γ-Aminobutyric acid; bicuculline; picrotoxin; internal perfusion; competition blockade; channel blockade; frog dorsal root ganglion.

The use of internal perfusion techniques[3] on isolated neurons allows a more rigorous electrophysiologic analysis of transmitter and antagonist actions than has usually been possible. We have applied such methods to study the mechanisms of antagonism of GABA responses on bullfrog dorsal root ganglion cells by bicuculline and picrotoxin.

Materials and methods. Bullfrog lumbar dorsal root ganglion cells (primary afferent neurons) were prepared for internal perfusion as previously described[3]. To suppress the ionic currents due to ions other than Cl^- we substituted in both the internal and external perfusion solutions $TRIS^+$ for Na^+, Ca^+ for K^+, and Mg^{2+} for Ca^{2+}. The composition of these solutions is given in the table. γ-Aminobutyric acid (GABA) and/or its antagonists were added to the appropriate solutions at known concentrations. GABA was perfused at 10-min intervals. The voltage-clamp circuit used was a modification of the single-electrode clamp of Wilson and Goldner[4].

Ramp current-voltage (I–V) relations were obtained at rest, at the peak response after GABA perfusion and at the peak GABA response after perfusion of the antagonist for 10 min extracellularly or 15 min intracellularly. At low GABA concentrations all I–V curves were taken during the plateau of the response. At higher GABA concentrations the response would peak and then fall during perfusion as a result of receptor desensitization[3]. In such cases the I–V plot was obtained at the peak response as possible. Patch clamp studies were done as described by Hamill et al.[5].

Results and discussion. When bicuculline or picrotoxin was applied to the external perfusion medium each inhibited the cell's response to GABA. Relative conductance showed a sigmoidal increase with increasing GABA concentration, with a half-maximal value at 4.6×10^{-5} M (fig. 1a). After complete recovery by washing with the control solution, the preparations were pretreated with an antagonist for 10 min, followed by application of a test solution containing an antagonist and GABA. Bicuculline (10^{-5} M) produced a parallel curve shifted to the right, without altering the maximal conductance, $\bar{g}_{max}$, – a shift characteristic of competitive inhibiton. Picrotoxin depressed $\bar{g}_{max}$ but did not change the concentration of GABA producing a half-saturation response – a curve indicating noncompetitive inhibition.

I–V relations in the presence of GABA with and without bicuculline or picrotoxin are shown in figure 1b, C. In the presence of GABA alone the relation was nonlinear, presumably reflecting a decrease in the mean channel open time with hyperpolarization, as has been observed for the glutamate-mediated excitatory channels[6] and also for the GABA-activated Cl^- channels of locust muscle fibers[7]. While bicuculline caused a considerable reduction in membrane conductance, it did not alter the voltage dependence (fig. 1b). In contrast, the nonlinearity disappeared in the presence of picrotoxin (fig. 1c). Such a voltage dependence of antagonism may indicate channel blockade[8]. The difference in voltage dependence between these 2 GABA antagonists is consistent with the conclusion that they act at different sites.

If picrotoxin acts at the level of the Cl^- channel, it might be expected to block when applied internally. Figure 2A shows the currents recorded in a cell externally perfused with 10^{-5} M GABA before, during and after a 15-min internal perfusion with 10^{-5} M picrotoxin. Internal picrotoxin caused a reversible reduction of the GABA current. In contrast, internal perfusion of 10^{-5} M bicuculline had no effect on the GABA current.

Composition of perfusing solutions

	Extracellular	Intracellular
Control		
NaCl	112 mM	–
KCl	2	30 mM
$CaCl_2$	2	–
Glucose	5	–
K-aspartate	–	100
EGTA	–	0.5
Na^+, Ca^{2+}, K^+-free		
TRIS-Cl	83	–
$CsCl_2$	2	35
$MgCl_2$	5	–
TEA-Cl	23	25
4-AP	3	–
Glucose	5	–
Cs-aspartate	–	70
EGTA	–	0.5