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THE INHIBITORY ACTION OF OESTRADIOL-17-β AND PROGESTERONE ON VENOUS SMOOTH MUSCLE

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1 The *in vitro* action of oestradiol-17-β (0.1, 1.0 and 10.0 μg/ml) and progesterone (0.1, 1.0 and 10.0 μg/ml) on the spontaneous activity of the portal veins in female rats at different stages of gestation was studied.

2 Progesterone caused the spontaneous mechanical activity in the rat portal vein to decrease in amplitude and increase in frequency. This action was dose-dependent and the sensitivity of the tissue decreased throughout pregnancy.

3 Oestradiol-17-β had a biphasic effect on spontaneous mechanical activity in the rat portal vein. At 0.1 μg/ml the vessel was stimulated while a similar effect to progesterone occurred with higher concentrations. The tissue was more sensitive to oestradiol at 7 days of gestation than at either the 10-14 or 17-21 day periods of gestation.

4 These effects of oestradiol and progesterone were still seen after blockade of the β-adrenoceptors.

5 Hydrocortisone (10 μg/ml) had no inhibitory effect on the spontaneous mechanical activity of the vein.

6 The veins from the 17-21 days pregnant animals showed a smaller amplitude of contraction than comparable non-pregnant females.

Introduction

Virchow (1856) made the observation that there were three major factors to be considered in the pathogenesis of venous thrombosis. These were, damage to the endothelium, an increase in blood coagulability and stasis of blood in the veins. Varicosity is a condition which could cause stasis of the blood in the veins thus contributing one factor towards Virchow's 'triad'. Wessler (1962) showed that when blood was mechanically stopped in superficial veins there was some thrombus formation after 30 minutes. Thus stasis is a powerful factor contributing towards thrombosis.

The occurrence of varicose veins and thrombophlebitis during pregnancy (Aaro & Juergens, 1971) and during oral contraceptive therapy (Grant, 1969) suggests that the steroids oestradiol-17-β and progesterone (which are elaborated in large amounts during pregnancy) may be responsible. The present experiments were to test the theory that these hormones directly cause inhibition of the venous smooth muscle and consequent dilatation of veins. The mechanism of inhibition was also investigated.

The tissue chosen for this investigation was an *in vitro* preparation of the rat portal vein. Thrombosis of mesenteric veins has been reported in conjunction with the use of orally administered steroids (Bergain, 1969; Hurwitz, Martin, Grossman & Waddel, 1970). Thus, any findings can be used to assess the role of the steroids in the aetiology of this type of complaint.

Methods

White Wistar rats weighing between 200-350 g were killed with a sharp blow on the back of the skull. The abdomen was then opened and the portal vein was located and exposed. Ligatures were placed around the vein at the point of its entry into the liver and at its junction with the superior mesenteric vein. This portion of the vein along with the ligatures was 2.5-3.0 cm long and was removed to a beaker containing modified Krebs (1950) solution of the following composition (mM): NaCl 118, KCl 4.69, $NaHCO_3$ 25.0, NaH_2PO_4 1.33, glucose 5.56, $CaCl_2$ 2.52 and

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$MgCl_2$ 1.05. The vessel was gently cleared of surrounding adipose tissue and was mounted vertically in a 10 ml organ bath. The bath was perfused at 15-20 ml/min with fresh Krebs solution which was prewarmed to 37° C and pre-oxygenated with 95% O_2 and 5% CO_2. The bath was surrounded by a water jacket through which water at 37° C was pumped.

The vein was stretched and relaxed two or three times and finally stretched until its length was approximately the *in vivo* length and the tension was approximately 0.25 g (2.5 mN). A period of equilibration was then allowed while the muscle relaxed and began to contract spontaneously. These contractions were recorded with an isometric force transducer and a pen recorder (Devices). Usually 30 min was long enough to allow a regular pattern of activity to be established. Four separate series of experiments were carried out with this preparation. Their protocols are described below.

(A) *Oestradiol and progesterone*

Thirty-four experiments were carried out in this group. The portal vein was removed and set up as previously described. After the initial period of equilibration the spontaneous mechanical activity was recorded during the following four consecutive periods: (i) 30 min–Normal Krebs perfusate; (ii) 2 h–0.1 μg/ml steroid perfusate; (iii) 2 h–1.0 μg/ml steroid perfusate; (iv) 2 h–10 μg/ml steroid perfusate. This protocol was used because it was difficult to reverse the effect of the steroid by washing with normal Krebs solution. In order to test three concentrations of steroid on each preparation and still keep the experimental time short this form of cumulative dose response trial was used. Ethanol (1 ml/l) was used throughout to make the steroids water soluble.

The wet weight of each vein was determined at the end of each experiment and all measurements of force produced by each preparation were expressed as milliNewtons/mg wet weight of tissue. (10 mN ≙ 1 g).

The frequency of contraction and the average amplitude of contraction/mg of tissue were measured during the last 10 min of each experimental period. The total contractile activity was estimated by integration of the trace of contractions against time. This was useful as an overall indicator of total activity in that an increase in the frequency of contraction, the amplitude of contraction or the duration of contraction produced an increase in the integral. The calculation was done automatically with a Resistance-Capacitor operational amplifier. The input to the integrator was balanced so that the resting tension in the preparation did not affect the integral. The capacitor was automatically discharged every minute.

Twelve young anoestrous female rats were used as a baseline group. Seven of these were used in a progesterone experiment and five in an oestradiol experiment. The remaining 22 animals were used for experiment at stages of pregnancy. Vaginal washings were examined daily and the day when sperm were found was designated as day 1 of pregnancy. Six animals were used at 7 days of gestation (3 for progesterone and 3 for oestradiol). Eight animals were used at 10-14 days of gestation (5 for progesterone and 3 for oestradiol). Eight animals were used at 17-21 days of gestation (5 for progesterone and 3 for oestradiol).

The effects of the steroids was assessed by noting the changes produced in the amplitude of contraction, the average frequency of contraction and the total contractile activity. The significance of any of these changes was tested by a paired *t* test.

The effects of pregnancy were assessed by noting the differences between these measurements during the initial 30 min perfusion period in Krebs solution over the four groups of animals. The significance of any difference was tested with a *t* test.

(B) *The controls*

To establish that any change in spontaneous activity of a preparation was due to the steroid perfusate and not due to temporal changes in the muscle or to ethanol, a total of eight control experiments were carried out with rats from each of the pregnancy groups and the portal veins were removed and set up as previously described. The spontaneous mechanical activity was recorded while the bath was perfused with fresh Krebs solution plus 1 ml/l of steroid-free ethanol. This was often done concurrently with a steroid experiment and the measurements and calculations were made in the same way.

(C) *The effects of β-adrenoceptor blockade*

Six experiments were carried out in this section. Oestradiol was used in three of these and progesterone in the others. The effects of isoprenaline (Boots) were first tested by injecting known amounts (1 μg and 10 μg) of the drug into the bath along with the perfusate. Washing out was automatic because the bath was continuously perfused with fresh Krebs solution. When the effects of β-adrenoceptor stimulation had been observed, the perfusate was changed to one

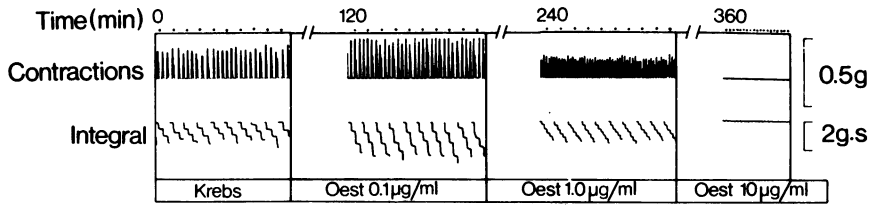

Figure 1 The spontaneous mechanical contractions and the minute integral of the portal vein from a young non-pregnant female rat. The block of records on the left shows the pattern of activity after equilibration in Krebs solution. The other three blocks show the effects of oestradiol (Oest) in concentrations of 0.1, 1.0 and 10.0 µg/ml when added cumulatively for periods of 2 h each.

containing propranolol 1.0 µg/ml (I.C.I.) and the tissue left for 30 minutes. β-Adrenoceptor blockade was confirmed by challenging the tissue with the same amounts of isoprenaline. Then either progesterone (10 µg/ml) or oestradiol (10 µg/ml) was added to the perfusate and the effect on the spontaneous mechanical activity was observed.

(D) *The effects of hydrocortisone*

Three experiments were carried out in this group. After a period of equilibrium and recording of normal activity, hydrocortisone (10 µg/ml) was added to the organ bath perfusate and the spontaneous activity was observed for two hours.

Results

The effects of oestradiol and progesterone on the spontaneous mechanical activity of portal veins from non-pregnant rats

Oestradiol-17-β Figure 1 shows the effect of cumulative addition of oestradiol in concentrations of 0.1, 1.0 and 10.0 µg/ml to the bath containing a rat portal vein. This portal vein was taken from a young anoestrous female rat. On the left of the figure is a block of records taken while the tissue was exposed to normal Krebs solution. A time base with one pulse per min is shown at the top. The trace beneath the time base shows the spontaneous contractions of the tissue as an upwards deflection of the pen. The integral of this contraction/time curve was determined electronically and is shown on the bottom trace. The integral is an estimate of the area under the curve and the total contractile activity of the tissue. This value is shown as a downwards deflection of the bottom trace and is reset to zero every minute. The three blocks of recording to the right of the first are similar records of the portal vein contractions and the integral of the contractions taken at the end of three consecutive 2 h periods when perfusates containing oestradiol at 0.1, 1.0 and 10.0 µg/ml were used. Calibrations for the pen deflections are shown on the extreme right and apply to all traces on the figure.

Figure 1 shows that during the initial period of Krebs perfusion there was a regular pattern of activity. The average amplitude of contraction was approximately 0.2 g and these occurred at a frequency of 2-3/minute. The integral on the lower trace shows that there was very little variation in the total contractile activity, the average in each minute being approximately 1.5 g.second. When the tissue was exposed to a perfusate containing 0.1 µg/ml of oestradiol for 2 h, the pattern of activity changed to that shown in the second block of traces. There were increments in the amplitude of contraction and the frequency of contraction. The integral shows that there was an increase in the total contractile activity to approximately 2.5 g.second. The third block of records in Figure 1 shows the activity after a 2 h period of exposure to oestradiol at 1.0 µg/ml. At this concentration a reduction in the amplitude of contraction to below the initial value in Krebs solution occurred and although the frequency of contraction simultaneously increased, the resultant total contractile activity in each minute was much reduced to approximately 1.0 g.second. A further increase in the concentration of the oestradiol to 10.0 µg/ml caused further large reductions in the activity of the muscle. The fourth block of records shows that after exposure to 10.0 µg/ml oestradiol for two hours the activity is of low amplitude but high frequency. Thus oestradiol seems to have a biphasic effect. At low concentrations the muscle of the rat portal vein is stimulated to stronger contraction. After 2 h of oestradiol at 1.0 µg/ml there was a significant decrease in the average amplitude of contraction ($P < 0.05$), an increase in the number of contractions ($P < 0.05$) and a decrease in total

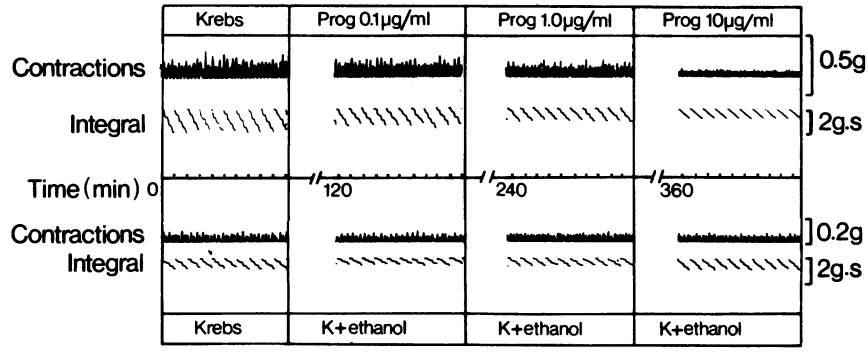

Figure 2 The spontaneous mechanical contractions and the minute integral of two portal veins from two young non-pregnant female rats. Above the time base are shown the records when progesterone (Prog) at concentrations of 0.1, 1.0 and 10.0 μg/ml were added cumulatively for periods of 2 h each. Below the time base are shown the records when no steroid was added to the bath. This was recorded simultaneously with the progesterone experiment above and a perfusate of Krebs plus ethanol (1 ml/litre) was used throughout.

contractile activity ($P < 0.01$). These effects were further accentuated by the use of oestradiol at 10 μg/ml for a further 2 h ($P < 0.01$). These P values are based on 5 experiments with anoestrous rats.

Progesterone Figure 2 shows two experiments similar to the previous oestradiol experiment. Above the time base (one pulse/min) are shown the records when progesterone at concentrations of 0.1, 1.0 and 10.0 μg/ml were cumulatively perfused through the organ bath for 2 h each. The records on the extreme left show the contraction pattern and the minute integral when the tissue was exposed only to Krebs solution. The average amplitude of contraction was approximately 0.5 g. and these occurred 3-4 times per minute. The integrals show that the total contractile activity produced was between 1.5-2.0 g.second. When progesterone at a concentration of 0.1 μg/ml was introduced to the tissue bath the contractile activity did not noticeably change. The effects of progesterone 1.0 μg/ml are shown in the third group of records. There was a noticeable decrease in the amplitude of the contractions and an increase in the frequency of contraction. The integral showed that overall there was a decrease in the total contractile activity. Further elevation of the progesterone concentration to 10.0 μg/ml caused the same effects but to a greater degree.

Seven experiments were carried out when cumulative dose responses of portal veins from anoestrous female rats to progesterone were investigated. After 2 h of exposure to 0.1 μg/ml there was no significant change in any of the variables. An increase in the dose to 1.0 μg/ml caused a significant reduction in the average amplitude of contraction ($P < 0.05$), a significant increase in the number of contractions ($P < 0.05$) and a significant decrease in total contractile activity ($P < 0.05$). These effects were further accentuated by the use of progesterone at 10 μg/ml for a further 2 h ($P < 0.01$).

Controls No attempt was made in either the oestradiol or progesterone experiments of Figures 1 and 2 to return the tissue to its orginal level of activity by washing with a steroid-free perfusate. Also no account was taken of the possible independent effects of ethanol (1 ml/l) which was used to make the steroids water soluble. Therefore a series of control experiments was carried out to show that the tissue was capable of sustaining regular activity without any change in amplitude or frequency when Krebs solution plus steroid-free alcohol was perfused for seven hours. One such experiment is shown below the time base in Figure 2. It was simultaneously carried out with the progesterone experiment above the time base and shows very little change in the pattern of activity over the same period as the concurrent progesterone experiment. Thus any change observed in the spontaneous activity of the portal veins was not due to temporal deterioration of the tissue or to ethanol.

Eight of these control experiments were carried out. No significant changes were observed after 4 h of perfusion with Krebs plus steroid-free alcohol ($P > 0.05$). After 6 h the only variable observed to change was the average amplitude of contraction. This showed a decrease from 0.59 ± 0.05 mN/mg to 0.42 ± 0.00 mN/mg and this was found to be

significantly different ($P < 0.05$). These control experiments show that there is some temporal deterioration of the tissue. However this deterioration was small and occurred simultaneously with the enormous decrease in activity caused by 10 μg/ml of steroid. There was no temporal deterioration before that. Thus the changes which occurred during consecutive exposure of a rat portal vein to increasing concentrations of either oestradiol or progesterone were not due to temporal deterioration of the tissue but to the changes in the perfusate.

Pregnant rats–sensitivity to oestradiol and progesterone

Oestradiol and progesterone were applied in similar cumulative concentrations to the portal veins of rats at various stages of gestation. The same variables were measured and the results at the end of each 2 h period of steroid perfusion were expressed as a percentage of the value in the initial 30 min control period when Krebs was used. Figures 3, 4 and 5 show these percentage results averaged over a number of experiments at different stages of gestation.

Total contractile activity Figure 3 shows the total contractile activity after progesterone or oestradiol. The dose-response curves for progesterone are shown on the left and those for oestradiol on the right. The closed circles represent the results from the non-pregnant animals. The open circles represent the results from the 7 day pregnant animals. Open squares represent the results from the 10-14 day pregnant animals and the closed squares represent the 17-21 day pregnant animals.

The progesterone curves (Figure 3a) show that the steroid produced a decrease in the total contractile activity which is dose-dependent at all times during gestation. The curves for the non-pregnant, 7 day and 10-14 day pregnant animals are overlapping, indicating no difference in sensitivity of the venous muscle to the steroid. The curve found for the animals at the end of pregnancy was displaced to the right of this group. This indicates that there was a decrease in the sensitivity of the tissue at this time.

The oestradiol curves (Figure 3b) show that oestradiol has a biphasic effect. At a concentration of 0.1 μg/ml all groups of animals demonstrate an increase in total contractile activity. This was reversed after 1.0 μg/ml and a marked decrease to almost zero was produced after 10.0 μg/ml. Peak sensitivity to the oestradiol was exhibited by the animals which were 7 days pregnant (open circles).

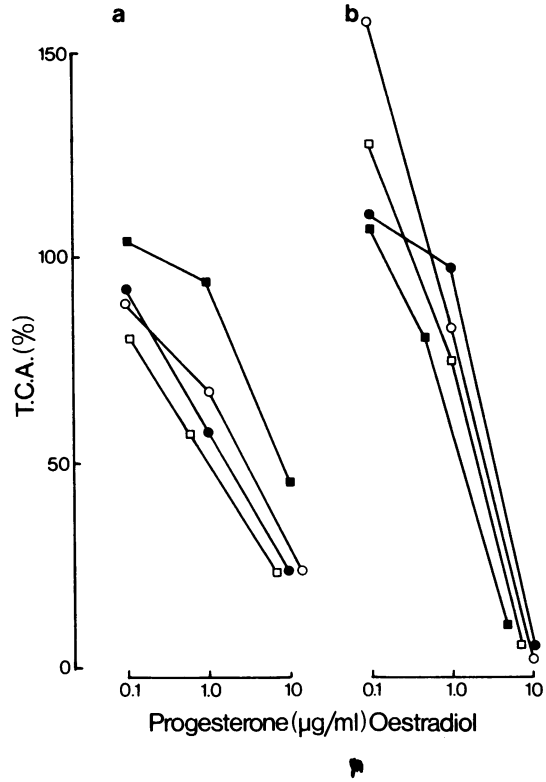

Figure 3 Total contractile activity (T.C.A.)—the % value was obtained by calculating 100 x T.C.A. (steroid)/T.C.A. (presteroid). Dose-response curves are shown for the portal veins from non-pregnant rats (closed circles), 7 days pregnant rats (open circles), 10-14 days pregnant rats (open squares) and 17-21 days pregnant rats (closed squares).

Average amplitude of contraction Figure 4 shows the changes caused by progesterone and oestradiol in average amplitude of contraction at various stages of pregnancy. The dose-response curves are again expressed as a percentage of control values in Krebs solution.

The progesterone curves (Figure 4a) show a dose-dependent decrease in the average amplitude of contraction. The sensitivity seems to be different at different stages of gestation. The non-pregnant and 7 days pregnant animals have similar sensitivities. The 10-14 day pregnant animals show an increased sensitivity while the 17-21 day pregnant animals show a decreased sensitivity.

The oestradiol curves (Figure 4b) show a biphasic response similar to that for total contractile activity. The time of greatest sensitivity was again at 7 days of gestation.

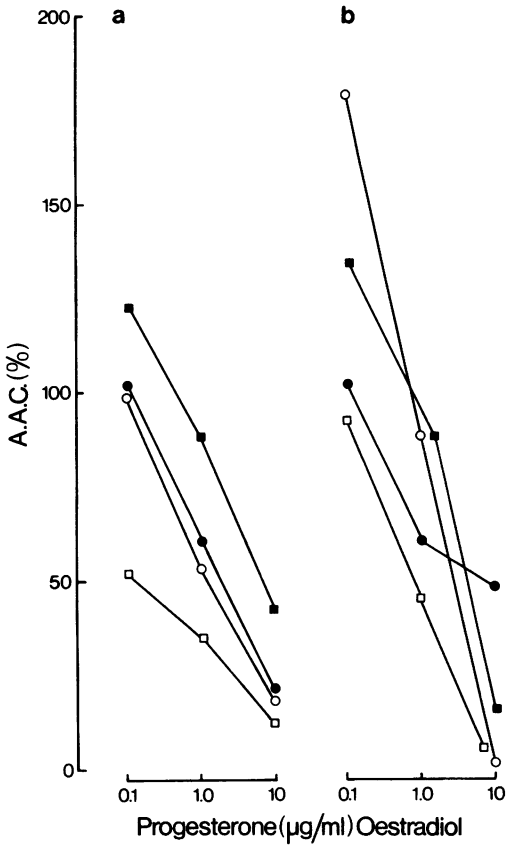

Figure 4 Average amplitude of contraction (A.A.C.)—the % value was obtained by calculating 100 x A.A.C. (steroid)/A.A.C. (presteroid). Dose-response curves are shown for the portal veins from non-pregnant rats (closed circles), 7 days pregnant rats (open circles), 10-14 days pregnant rats (open squares) and 17-21 days pregnant rats (closed squares).

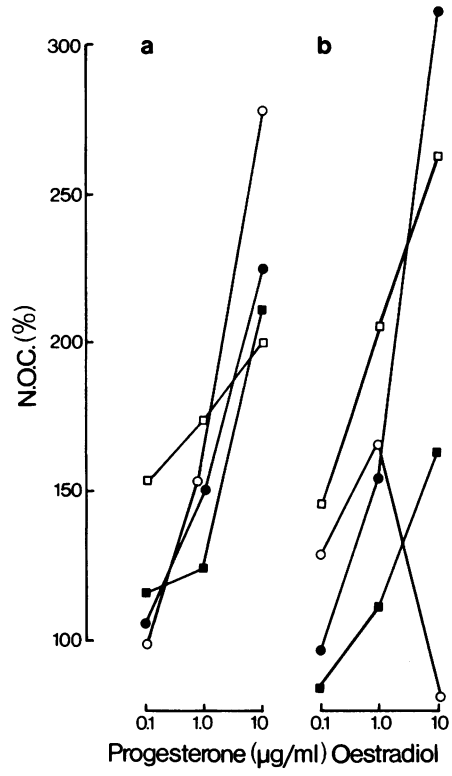

Figure 5 Number of contractions (N.O.C.)/10 minutes—the % value was obtained by calculating 100 x N.O.C. (steroid)/N.O.C. (presteroid). Dose-response curves are shown for the portal veins from non-pregnant rats (closed circles), 7 days pregnant rats (open circles), 10-14 days pregnant rats (open squares) and 17-21 days pregnant rats (closed squares).

Number of contractions/10 minutes Figure 5 shows similar dose-response curves for the effect of progesterone and oestradiol on the number of contractions per 10 minutes.

The progesterone curves (Figure 5a) show that there is an increase in the frequency of contraction after progesterone and that this effect was dose-dependent. The oestradiol curves (Figure 5b) show a similar pattern. With one exception, an increase in the rate occurred after oestradiol and this increase was dose-dependent. The exception was seen after oestradiol 10 μg/ml in the group of animals which were 7 days pregnant. A decrease in the rate of contraction to zero occurred at this point.

From Figures 3, 4 and 5, it is evident that pregnancy modifies the response of the rat portal

Table 1 Venous activity changes during pregnancy

	Nonpreg	*17-21 days preg*	*Significance*
Amplitude of contraction (mN/mg)	0.67 ± 0.06	0.53 ± 0.07	$P < 0.05$
Number of contractions/10 min	39.57 ± 2.67	36.0 ± 4.96	NS
Total contractile activity mN/mg	87.6 ± 7.9	68.1 ± 9.5	$P < 0.05$

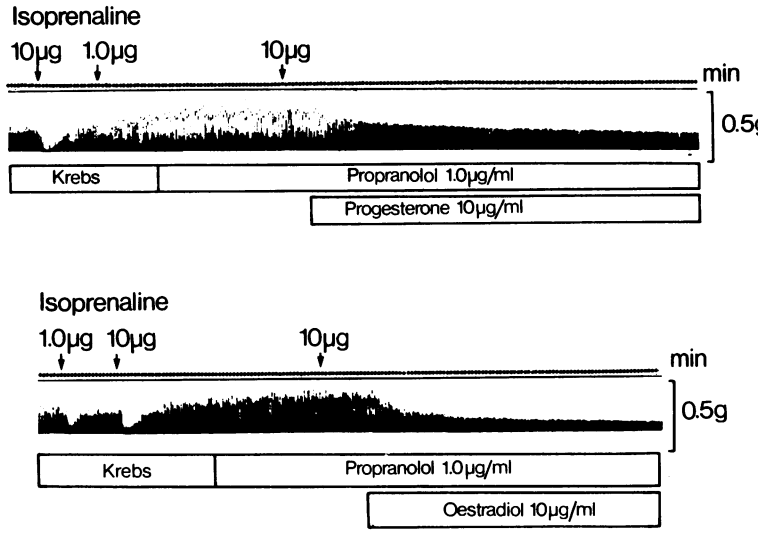

Figure 6 The spontaneous mechanical activity of a rat portal vein when 10 μg/ml progesterone (upper trace) or 10 μg/ml oestradiol (lower trace) was used after β-receptor blockade with propranolol (1 μg/ml). Contraction and time calibrations are shown.

vein to the steroids. The sensitivity to progesterone was similar in the non-pregnant and 7 days pregnant groups. The 10-14 days pregnant group showed slight hypersensitivity while the final 17-21 days pregnant group was hyposensitive. The maximum sensitivity to oestradiol occurred in the 7 days pregnant group.

Initial levels of spontaneous activity As well as changing the sensitivity to the steroids pregnancy seemed to have an influence on the initial amplitude of activity when normal Krebs was used as a perfusate. Table 1 shows the amplitude of contraction, number of contractions and the total contractile activity in the non-pregnant and 17-21 days pregnant groups. The average amplitude of contraction and the total contractile activity were significantly lower in the pregnant than non-pregnant group ($P < 0.05$). There was no significant change in the number of contractions/10 minutes ($P < 0.25$).

Thus pregnancy seems to modify both the spontaneous activity of the rat portal vein and the sensitivity of this preparation to progesterone and oestradiol-17-β.

The effects of β-adrenoceptor blockade

Figure 6 shows two records of the spontaneous mechanical activity of two rat portal veins. The upper trace shows the activity when progesterone (10 μg/ml) was used. At the beginning of the experiment when normal Krebs solution was being used, isoprenaline (10 μg and 1 μg) caused inhibition of the spontaneous activity by stimulation of the β-adrenoceptors. After 30 min of perfusion with propranolol (1 μg/ml) these receptors were shown to be blocked because isoprenaline (10 μg) had no effect. When progesterone (10 μg/ml) was used its inhibitory effect on the amplitude of contraction was still evident. The lower trace shows a similar record when oestradiol (10 μg/ml) was used after β-blockade. Inhibition of the amplitude of contraction was still present. The same result occurred in all six experiments which were carried out.

The effect of hydrocortisone

The spontaneous mechanical activity of a rat portal vein before and after exposure to hydrocortisone (10 μg/ml) was also measured. Unlike oestradiol or progesterone, hydrocortisone caused no apparent inhibition of the muscle. This result was found in each of the three experiments which were carried out.

Discussion

The rat portal vein carries blood from the intestine, stomach and spleen to the liver. Thus the blood in the vein has already been through one

microcirculation and must be at a much reduced hydrostatic pressure. Normally the movement of blood in the peripheral venous system is helped either by pressure exerted externally during contraction of surrounding skeletal muscles which forces blood through a one way system of valves, or by negative pressure from the abdomen. Neither of these mechanisms can be applied to the hepatic portal vein because it is surrounded by fatty tissue of the abdomen and hence cannot have an external muscle pump. Secondly, it runs between two sets of capillaries and it would seem unlikely that negative pressure in the inferior vena cava would be transmitted through the liver to the portal vein. One possible mechanism for the movement of blood along the vein could be the inherent spontaneous activity of the smooth muscle wall in the vessel. This spontaneous contractile activity could be functioning as an intrinsic muscle pump. By inhibiting this spontaneous activity the blood in the vessel is left only with the driving force of hydrostatic pressure transmitted through the intestinal capillaries. The evidence presented in the present experiments demonstrates that the steroids oestradiol-17-β and progesterone are capable of inhibiting the spontaneous activity of the longitudinally arranged muscle fibres in the rat portal vein. In conjunction with an inhibition of the circularly oriented fibres stasis of blood would therefore result from *in vivo* administration of oestradiol or progesterone. The stasis of blood flow in conjunction with the steroid-induced hypercoagulability (Phillips, 1963) is proposed as a possible cause of the cases of mesenteric venous thrombosis described by Berjain (1969) and Hurwitz *et al.* (1970).

Natural sources of progesterone and oestradiol are the ovary and the placenta. During pregnancy large amounts are produced, particularly by the placenta, and the systemic blood concentration of the steroids is higher. The comparisons of portal veins taken from groups of rats at different stages of gestation was an attempt to see if this naturally elevated blood steroid level would have an effect on the spontaneous activity of the vein. The results showed that there was a significant difference in the spontaneous activity between the portal veins from the non-pregnant group and the veins from the 17-21 days pregnant group. Kuriyama & Csapo (1961) showed that strips of rabbit uterine muscle taken at different stages of gestation had markedly different mechanical properties. At the beginning of the pregnancy the muscle strip was spontaneously active and when stimulated all the strip contracted. At the end of the pregnancy this situation was reversed. The muscle was mechanically and electrically quiescent and there was no propagation of induced contraction down the tissue. The present experiments indicate that a similar reduction in the spontaneous activity in the portal vein of the rat occurs during pregnancy. Kuriyama & Csapo (1961) implicated progesterone as the cause of the uterine quiescence. The possibility that the steroids were involved in the portal venous inhibition seems likely, as they caused this effect *in vitro*.

The present results show that a change in the activity of the portal vein occurs at the end of a pregnancy. The systemic blood levels of oestrogens at this time have been shown to be less than 1 ng/ml (Yoshinaga, Hawkins & Stocker, 1969). Progestins have however been measured at 0.13 μg/ml (Hasimoto, Henricks, Anderson & Melampy, 1968). Although these concentrations have not been shown to be effective in the present acute experiments the possibility exists that chronic exposure to the circulating steroid (particularly progesterone) is effective in producing the recorded changes in activity between the non-pregnant and the 17-21 days pregnant group. Sensitivity of the veins to added oestradiol and progesterone was greatest with vessels removed at the beginning of pregnancy. The circulating steroids may have caused accommodation of the venous muscle and hence as pregnancy progressed the action of the added steroids becomes smaller. Oestradiol throughout was more effective than progesterone and instead of a single inhibitory effect it displayed a biphasic effect. At low concentrations, oestradiol appeared to stimulate the muscle to greater activity, but at all higher concentrations caused the inhibition and acceleration of the vessel. Normally, the steroids caused the inhibition of the amplitude of contraction, but acceleration of the frequency of contraction. If these steroids had the same effect on the venous muscle as progesterone has on the uterine strip (Kuriyama & Csapo, 1961) then the increase in frequency could be due to a decrease in the propagation of contraction through the tissue. Thus, instead of the venous muscle all contracting with a large amplitude in response to one unstable pacemaker cell, there are many such pacemaker cells producing small but frequent contractions. The increased frequency of contractions would then only be a stage towards the cessation of activity. This pattern was in fact found when oestradiol was applied to portal veins from rats which were 7 days pregnant (Figures 6, 7 and 8). Axelsson, Wahlström, Johansson & Jonsson (1967) found that a similar pattern occurred when the rat portal vein was artificially hyperpolarized using low potassium solutions as a bathing medium. The muscle was found to have a transient increase in frequency but decrease in amplitude of contrac-

tions. After 10 min all activity stopped. Oestradiol and progesterone were found by many workers to cause hyperpolarization of uterine muscle (Marshall, 1959; Goto & Csapo, 1959; Kuriyama & Csapo, 1961). In view of the findings of Axelsson *et al.* (1967) it seems likely that the mechanism of action of the steroids on the venous muscle is at least partly by hyperpolarization of the cell membrane. Oestradiol causes myometrial hyperpolarization and moves the membrane potential into the 'firing range' for spontaneous action potentials. The membrane potential of venous muscle is however already in its 'firing range'. Thus a low dose of oestradiol (0.1 μg/ml) must have moved the membrane potential into a more excitable area of this 'firing range' whereas the higher concentrations (1.0 μg/ml and 10 μg/ml) hyperpolarized the tissue out of this range completely. Progesterone causes uterine hyperpolarization and moves the membrane potential beyond the 'firing range'. Its action on venous muscle would be similar.

Shabanah, Toth, Carassavas & Maughan (1968) have suggested that the adrenoceptors of the uterus play a large part in the mediation of the effects of oestradiol and progesterone. Progesterone was proposed to act via the β-receptors to cause uterine quiescence during the pregnancy. β-receptor stimulation in the venous muscle caused much the same effects as the steroids. However, the present evidence shows that the two pathways are completely separate and that progesterone or oestradiol do not act via the β-receptors in venous muscle.

Thus these hormones have been shown to inhibit the activity of the muscle component of the vessel wall. Connective tissues have also been shown to be affected. Buckingham, Seldon & Danforth (1962) showed that connective tissues in the cervix were weakened during pregnancy and postulated that 'probably all connective tissue structures, not only the cervix undergo alteration and loss of tensile strength during pregnancy'. Later Danforth, Manalo-Estrella & Buckingham, (1964) found that the steroids oestradiol and progesterone did produce a similar loss of tensile strength in the connective tissue structures of many arteries and veins.

The steroid-induced weakness in the vein wall and the steroid inhibition of the smooth muscle must make the vessel more liable to dilate. In such a situation, an increase in venous blood pressure (due to the occlusion effects of an enlarging uterus) would be very effective in producing blood stasis and varicose veins. Thrombosis could quickly follow as the steroids have also been shown to produce a state of hypercoagulability in blood (Mishra & Mishra, 1969).

Thus the inhibitory action of oestradiol-17-β and progesterone on venous muscle has been demonstrated. A similar inhibition was found during the course of a pregnancy. It is postulated that these steroids act on the venous muscle in the same way as the myometrium and that the prediction of Kilbourne (1933) that there is a general loss of smooth muscle tone in all structures of the pregnant body may be correct, and may be due to the increased amount of circulating oestrogens and progesterone.

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