The Effect of Progesterone on Spontaneous and Agonist-Evoked Contractions of the Rat Aorta and Portal Vein

MARK S. MUKERJI, HELEN L. LEATHARD AND HENRY HUDDART*

*Department of Nursing Studies, St. Martin's College, Lancaster LA1 3JD, UK and
Department of Biological Sciences, IENS, Lancaster University, Lancaster LA1 4YQ, UK

Abstract

The mechanisms underlying the suppression of vasocontractility caused by progesterone were investigated by studying changes in the contractile force of rat isolated aorta and portal vein, induced by altering extracellular concentrations of noradrenaline (NA) potassium ions (K^+) and calcium ions (Ca^{2+}).

In the aorta, progesterone ($10\,\mu M$) had a general suppressive effect on NA-, Ca^{2+}- and K^+-induced contractions. In contrast, in the portal vein a more selective suppression of contractions was observed. Both tonic and phasic components of contractions induced by cumulative addition of Ca^{2+} to tissues equilibrated in Ca^{2+}-free saline were suppressed. The phasic but not tonic components of contractions induced by NA addition were suppressed. There was no significant effect on tonic contractions induced by elevated ($40–120\,mM$) K^+, but a concentration-dependent suppression of the phasic component of contractions was observed during depolarisation with smaller elevations of K^+ concentrations ($5–20\,mM$).

These results suggest that on the portal vein the suppressive effect of progesterone is due to a potassium channel opening action, whilst on the aorta a different or additional mechanism of suppression exists.

Progesterone is known to have a general suppressive effect on vascular smooth muscle contractility that seems physiologically more important than its additional, indirect vasoconstrictor effect (McCalden 1975; Eccles & Leathard 1985; Leathard & Eccles 1991; Jiang et al 1992; Mukerji et al 1995). Progesterone has been implicated in the suppression of postural vasoconstriction observed in-vivo by Hassan et al (1990) and as a vasoactive agent in the aetiology of menstrually related migraine (Leathard 1989). The following studies were designed to explore further the mechanism(s) of the inhibitory action of progesterone on vascular smooth muscle, specifically to differentiate between Ca^{2+} channel blockade (Jiang et al 1992; Glusa et al 1997) and K^+ channel activation (Leathard & Eccles 1987, 1991; Huyton & Leathard 1991).

The effect of progesterone was assessed on two different blood vessels from the rat, the aorta and portal vein, both commonly utilised in in-vitro experiments. They were selected on the basis of their differing functional roles in-vivo and the fact that previous investigators had attributed different mechanisms to progesterone's suppressive action on them.

The effect of progesterone on responses evoked by noradrenaline (NA), the principal neurotransmitter associated with the control of contractile activity in the aorta and portal vein, was assessed. Recent evidence indicates that α_{1A}-adrenoceptors mediate tonic contractions and α_{2A}-adrenoceptors mediate changes in the phasic activity of the portal vein. Contractions caused by NA occur via depolarisation-dependent and depolarisation-independent activation of voltage-operated Ca^{2+} channels, leading to entry of extracellular Ca^{2+}, and also via an increase in release of Ca^{2+} from intracellular stores (Mironneau et al 1996). It was therefore of interest to seek differing mechanisms of action of progesterone on the phasic and tonic components of contraction.

The dependence of vascular smooth muscle on extracellular Ca^{2+} (Ca^{2+}_o) for the requisite increase in intracellular Ca^{2+} differs between

Correspondence: H. L. Leathard, Department of Nursing Studies, St. Martin's College, Lancaster LA1 3JD, UK.

vascular smooth muscles (Allen & Bukoski 1982) and types of contraction (phasic or tonic) (Mironneau et al 1996). A direct study of the effect of progesterone on contractions of the aorta and portal vein induced by Ca^{2+}_o addition was planned to assess the effect of progesterone on Ca^{2+}_o-dependent contractions at basal membrane potential (E_m) conditions.

The action of progesterone on K^+-induced contractions was used to investigate the possibility of K^+ channel activation because the inhibition of contractions induced by low (10–30 mM) but not high (60–120 mM) K^+ concentrations has been observed for a number of known K^+ channel openers over a range of different vascular tissues, as described by Edwards et al (1992).

To provide comparative controls for progesterone the effects of two vasodilators, with different known mechanisms of action, were also assessed on the portal vein. Nifedipine has been shown to act via a voltage-operated Ca^{2+}-channel-blocking mechanism (Dacquet et al 1987), whilst minoxidil sulphate acts via a K^+-channel-opening mechanism (Newgreen et al 1989).

The putative mechanisms of progesterone's vascular effects discussed so far presuppose a non-genomic mode of action, as argued by Eccles & Leathard (1985) and Morishita (1986). This was re-examined simply by recording the speed of the onset effects of progesterone; seconds to minutes indicating a non-genomic mechanism of action, compared with hours indicating a genomic mechanism (Baulieu & Robel 1995).

These studies extend earlier research into mechanism(s) by which progesterone inhibits the contractility of different vascular smooth muscle by examining its effects on phasic and tonic contractions evoked by wide ranges of concentrations of external calcium and potassium and by NA in aorta and portal vein preparations from the rat.

Materials and Methods

Animals and equipment
Male Wistar albino rats weighing 300–400 g were obtained from the Lancaster University animal housing facility. The rats were stunned then killed by cervical dislocation. Sections of the portal vein (~2 cm in length) and thoracic aorta were immediately dissected out and placed in aerated Tyrode's mammalian saline. The portal vein was used intact. The aorta was spirally dissected to produce strips of tissue (~5 mm wide and ~2 cm in length). The tissues were suspended in conventional isolated organ baths (maintained at 37°C) under an initial tension equivalent to a load of 0·5–1·0 g. Changes in tension were recorded, via a Grass FT.03 isometric force displacement transducer, on a four-channel Grass Model 79 D Polygraph.

An initial equilibration period of 50–80 min was allowed. All test drugs and vehicles were added directly to the 10 mL organ baths, in volumes not exceeding 150 μL, and were washed out by replacing the Tyrode's saline. For both the aorta and portal vein the amplitude of changes in basal tone recorded 4 min after agonist addition were measured as the tonic component of contractions. For the portal vein, the phasic component of contractions was measured as the mean amplitude of phasic peaks recorded over a period of 3–5 min after agonist addition.

Experimental protocols
NA-induced contractions. Because of the prolonged recovery periods and desensitisation of the tissues observed utilising cumulative addition of NA, the effects of progesterone were examined on single additions of NA, at two concentrations selected from their respective NA cumulative addition response curves: 10 nm and 10 μM for the aorta; 0·2 and 50 μM for the portal vein.

Ca^{2+}-evoked contractions. The effect of progesterone was tested on contractions evoked by cumulative addition of Ca^{2+} (0·25–2·5 mM) to Ca^{2+}-free Tyrode's saline for the portal vein. For the aorta 'high K^+' saline (see below) was used in order to obtain contractions showing a graded increase in tension from 0·25 to 2·0 mM Ca^{2+}.

K^+-evoked contractions. Responsiveness to K^+ (20–80 mM) was assessed by constructing cumulative concentration–effect curves using a 6 min contact time for each dose addition of KCl to the saline. To establish the dose dependency of progesterone we utilised the K^+ depolarisation protocol to test a range of progesterone concentrations from 1 μM, the lowest concentration at which inhibition was observed in preliminary trials, to 30 μM, which causes complete inhibition of portal vein spontaneous activity (McCalden 1975). The initial concentration of progesterone examined (1 μM) was allowed 40 min equilibration with the tissue, after which the response to K^+ was re-examined in the continued presence of the hormone. After washout of the K^+ the tissue was re-equilibrated with a higher concentration of the hormone (10 μM) and the K^+ trial repeated. This protocol was carried out to give up to three consecutive hormone trials for

any given tissue. Concurrent time-matched controls were carried out on tissues exposed to the vehicle (ethanol) in appropriate concentrations and to Tyrode's saline. A considerable decrease in amplitude of contraction was observed over time with the saline controls. The effects of nifedipine ($0 \cdot 1 \, \mu M$) and minoxidil sulphate ($1 \cdot 0 \, \mu M$) were assessed on a single K^+ addition protocol.

Spontaneous phasic contractions. The effects of progesterone ($1-30 \, \mu M$), minoxidil sulphate ($1-10 \, \mu M$) and nifedipine ($0 \cdot 01-1 \, \mu M$) were assessed on the spontaneous activity of the portal vein by incubation of the respective agent for 40 min at basal resting conditions.

Drugs and solutions

Tyrode's saline had the following composition (mM): NaCl $137 \cdot 0$, KCl $5 \cdot 4$, $CaCl_2$ $1 \cdot 79$, $MgSO_4$ $1 \cdot 04$, NaH_2PO_4 $0 \cdot 34$, $NaHCO_3$ $11 \cdot 9$, glucose $5 \cdot 6$. It was maintained at $37^{\circ}C$ with a pH adjusted to $7 \cdot 25 \pm 0 \cdot 05$. Ca^{2+}-free Tyrode's saline omitted $CaCl_2$; 'high K^+' Ca^{2+}-free Tyrode's saline was made identically but with 55 mM KCl substituted for 55 mM NaCl. Ca^{2+} was added as $CaCl_2$. Where K^+ (as KCl) was used as a spasmogen the concentrations given include the K^+ ($5 \cdot 4$ mM) present in the normal Tyrode's saline. All drugs were obtained from Sigma. Progesterone and nifedipine were prepared in 70% ethanol as 10 mM and 1 mM stock solutions respectively.

Statistical analysis of results

The significance of differences between the effects of the hormone and those of the equivalent ethanol concentration were assessed using Student's t-tests for unpaired data; means were regarded as significantly different when $P < 0 \cdot 05$.

Results

NA-induced contractions

Aorta. NA added at two concentrations, 10 nM and $10 \, \mu M$, induced tonic changes in the tension of the aorta. Incubation with $10 \, \mu M$ progesterone caused significant inhibition of the tonic contractions: $62 \pm 10\%$ reduction of responses to 10 nM NA and $45 \pm 11\%$ reduction of responses to $10 \, \mu M$ NA (both $P < 0 \cdot 01$; n = 6) compared with ethanol controls.

Portal vein. NA added at two concentrations, $0 \cdot 2$ and $50 \, \mu M$, produced changes in both the basal tone and the phasic activity of the portal vein. Incubation with $10 \, \mu M$ progesterone had no significant effect on the tonic components of contractions induced by either concentration of NA, but there was significant suppression of the phasic components induced by both concentrations: $18 \pm 5\%$
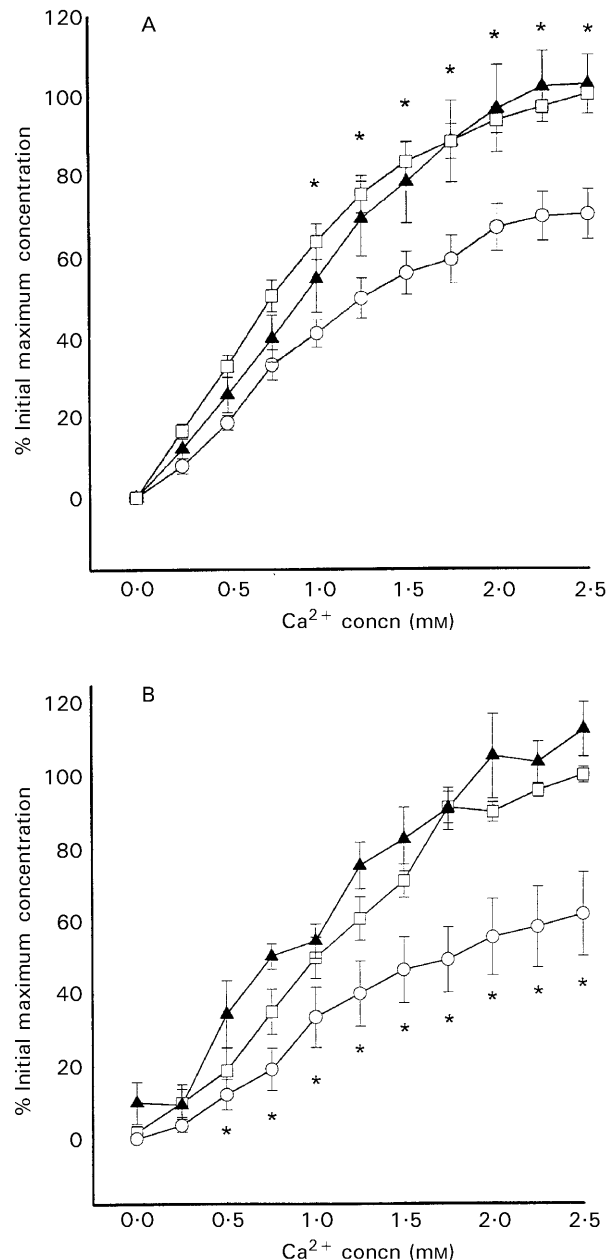

Figure 1. Inhibitory effects of progesterone (○) on tonic contractions of (A) rat aorta and (B) portal vein elicited by cumulative additions of Ca^{2+} ($0 \cdot 25-2 \cdot 5$ mM) to Ca^{2+}-free Tyrode's saline. Equivalent ethanol vehicle (▲) and saline-treated control (□) data are shown for comparison. *$P < 0 \cdot 05$ in Student's t-test between hormone and vehicle. Each point represents the mean $\pm$ s.e.m. of values recorded from n = 6 tissues.

inhibition of responses to $0.2\,\mu M$ NA ($P < 0.05$; $n = 8$) and $36 \pm 3\%$ inhibition of responses to $50\,\mu M$ NA ($P < 0.01$; $n = 8$) compared with ethanol controls.

Ca^{2+}-evoked contractions

Aorta. Cumulative addition of Ca^{2+} to a 'high K^+' Ca^{2+}-free saline caused a graded increase in tension from 0.25 to $2.0\,mM\ Ca^{2+}$. Progesterone appeared to inhibit all of the tonic responses to addition of Ca^{2+}, although this was only significant from 1.0 to $2.5\,mM\ Ca^{2+}$ (Figure 1). A $28.4 \pm 7\%$ decrease in tonic response was observed at $1.25\,mM$ Ca and a $31 \pm 6\%$ decrease at $2.0\,mM\ Ca^{2+}$ compared with ethanol controls.

Portal vein. Increasing Ca^{2+} concentrations progressively from 0 to $2.5\,mM$ caused a concentration-related increase in contractile activity of the portal vein. First tonic then phasic components of contractions gradually increased from zero at 0–$0.25\,mM\ Ca^{2+}$ to a maximum at 2.25–$2.5\,mM$ Ca^{2+}. Incubation with $10\,\mu M$ progesterone caused significant inhibition of the tonic component of contractions at all concentrations of Ca^{2+} from 0.5 to $2.5\,mM$: $36 \pm 12\%$ inhibition at $0.5\,mM\ Ca^{2+}$; $53 \pm 12\%$ inhibition at $1.25\,mM\ Ca^{2+}$; $53 \pm 10\%$ inhibition at $2.0\,mM\ Ca^{2+}$ and $55 \pm 10\%$ inhibition

at $2.5\,mM\ Ca^{2+}$ (Figure 1). The phasic component of contractions were concomitantly suppressed by $10\,\mu M$ progesterone: $40 \pm 5\%$ inhibition at $1.0\,mM$ Ca^{2+} ($P < 0.01$); $36 \pm 6\%$ inhibition at $1.5\,M\ Ca^{2+}$ ($P < 0.01$); $28 \pm 3\%$ inhibition at $2.0\,mM\ Ca^{2+}$ ($P < 0.05$) and $28 \pm 4\%$ inhibition at $2.5\,mM$ Ca^{2+} ($P < 0.05$) (all $n = 6$) compared with ethanol controls.

K^+-evoked contractions

Aorta. Increasing K^+ concentrations progressively from 5.4 to $120\,mM$ produced a characteristic response pattern consisting of graded increases in tonic contractions of the aorta. Progesterone ($10\,\mu M$) was observed to inhibit all of these tonic responses when compared with ethanol controls, although this was significant only from 40 to $120\,mM\ K^+$: $28 \pm 5\%$ inhibition at $40\,mM\ K^+$ and $34 \pm 7\%$ at $120\,mM\ K^+$ (Figure 2).

Portal vein. Increasing K^+ concentrations progressively from 5.4 to $80\,mM$ produced a characteristic response pattern on the portal vein consisting of graded increases, initially in phasic activity at $10\,mM\ K^+$, through to predominantly tonic contractions at $80\,mM\ K^+$. Incubation with progesterone (1, 10 and $30\,\mu M$) had no significant effect on the tonic component of contractions (Table 1), but there was a concentration-dependent suppression of the phasic component, as measured at $20\,mM\ K^+$: $19 \pm 3\%$ inhibition by $1\,\mu M$ progesterone, $52 \pm 11\%$ inhibition by $10\,\mu M$ progesterone and $65 \pm 13\%$ inhibition by $30\,\mu M$ progesterone compared with ethanol controls (Figure 3).

Comparative studies of the effects of other known vasodilators were also carried out, using the K^+ addition protocol, on the portal vein. Nifedipine ($0.1\,\mu M$) caused significant inhibition of the tonic component of contractions from 20–$80\,mM\ K^+$ (Table 1). The phasic component of contractions was also suppressed: $71 \pm 6\%$ inhibition ($P < 0.01$; $n = 3$–6) compared with the ethanol solvent control at $20\,mM\ K^+$. Minoxidil sulphate ($1.0\,\mu M$) had a similar effect to $10\,\mu M$ progesterone. No significant inhibitory effect was seen on the tonic component of contractions induced by K^+ addition (Table 1). There was, however, significant suppression of the $20\,mM\ K^+$-induced phasic component: $39 \pm 7\%$ inhibition ($P < 0.05$; $n = 3$–6) compared with the saline control.
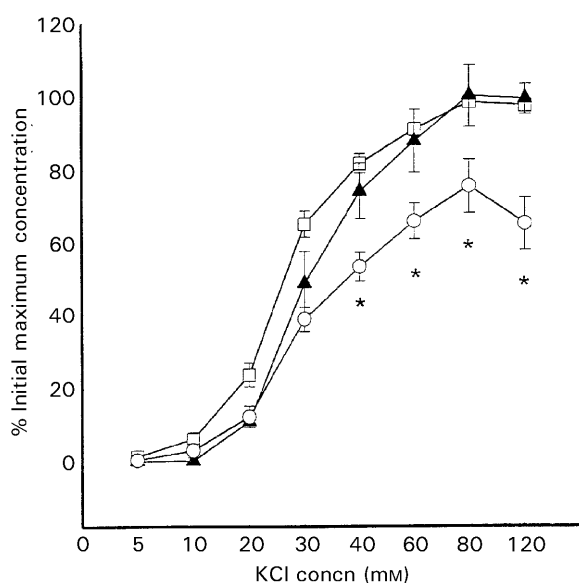

Figure 2. Inhibitory effects of progesterone (○) on tonic contractions of rat aorta elicited by cumulative additions of K^+ (10–$120\,mM$). Equivalent ethanol vehicle (▲) and saline-treated control (□) data are shown for comparison. *$P < 0.05$ in Student's t-test between hormone and vehicle. Each point represents the mean $\pm$ s.e.m. of values recorded from $n = 5$ (control), $n = 6$ (ethanol) and $n = 10$ (progesterone) tissues.

Spontaneous phasic contractions

The effects of progesterone, minoxidil sulphate and nifedipine on the spontaneous activity of portal

Table 1. Effect of progesterone, nifedipine and minoxidil sulphate on K^+-evoked tonic concentrations of the rat portal vein.

Hormone/solvent	20 mM K^+	40 mM K^+	80 mM K^+
Progesterone 1 μM (n = 5)	4.8 ± 0.7	39.6 ± 2.2	92.2 ± 4.1
Ethanol 20 mM (n = 13)	4.1 ± 0.8	39.3 ± 2.4	95.7 ± 2.6
P	0.53	0.93	0.49
Progesterone 10 μM (n = 6)	4.3 ± 1.1	29.5 ± 4.9	84.0 ± 6.1
Ethanol 20 mM (n = 7)	2.9 ± 1.4	26.6 ± 2.4	74.6 ± 12.0
P	0.42	0.61	0.50
Progesterone 30 μM (n = 6)	2.8 ± 0.8	28.2 ± 5.0	67.0 ± 8.5
Ethanol 20 mM (n = 6)	2.7 ± 1.7	22.8 ± 3.2	77.7 ± 5.8
P	0.93	0.40	0.33
Nifedipine 0.1 μM (n = 3)	3.0 ± 0.6	10.0 ± 0.1	23.0 ± 2.4
Ethanol 20 mM (n = 6)	6.6 ± 2.0	31.2 ± 5.0	88.6 ± 4.0
P	0.15	0.03	0.02
Minoxidil sulphate 1.0 μM (n = 3)	5.8 ± 1.8	51.3 ± 6.9	95.8 ± 5.3
Saline (n = 6)	9.5 ± 2.6	61.7 ± 5.2	99.0 ± 0.8
P	0.19	0.40	0.26

Values given for progesterone, nifedipine, minoxidil sulphate and their solvents are the amplitudes of 20–80 mM K^+-evoked contractions of portal vein expressed as percentages of the initial maximum response of the preparation to 80 mM K^+. Solvent data correspond to a specific time-matched incubation for the solvent, equivalent to that of the related drug (and concentration) tested. P values were calculated using Student's t-test for unpaired data to give statistical comparisons between the drug and its solvent.

vein were compared. Progesterone caused an initial decrease in amplitude after 5 min (approx.) but a concomitant increase in frequency at 1.0 and 10.0 μM After 30 min (approx.) incubation with 10.0 μM progesterone both amplitude and frequency usually decreased. Progesterone (30 μM)
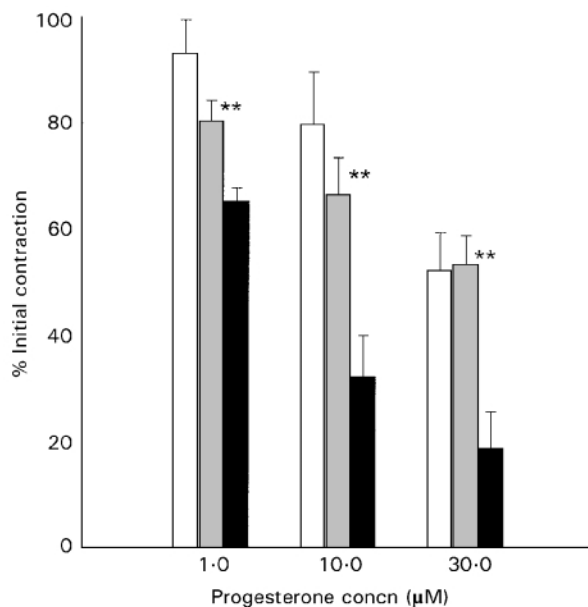

Figure 3. Concentration-dependent inhibitory effects of progesterone (■) on the phasic component of 20 mM K^+-evoked contractions of rat portal vein. Equivalent time-related decrements of phasic contractions in ethanol (▨) and saline-treated control (□) tissues are also shown. *$P < 0.05$, **$P < 0.01$ in Student's t-test between hormone and ethanol vehicle. Each point represents the mean ± s.e.m. of values recorded from n = 5–7 tissues.

caused a decrease in the amplitude and frequency of spontaneous contractions, which often resulted in a complete cessation of spontaneous activity. A similar pattern of change in activity was observed with both minoxidil sulphate and nifedipine. Minoxidil sulphate (1 μM) and nifedipine (0.01 μM) caused a slight decrease in amplitude but an increase in frequency and minoxidil (10 μM) sulphate and nifedipine (0.1–1.0 μM) caused decreases in both amplitude and frequency that eventually caused a cessation of spontaneous activity.

Onset of inhibition post-addition of drug/vehicle to the organ bath was measured as the time at which a 20% reduction in phasic activity of the portal vein had been reached. Progesterone (10 μM) effected this reduction in 3.4 ± 0.5 min ($P < 0.01$; n = 8) compared with the equivalent volume of ethanol vehicle, which took 25.2 ± 3.4 min. Saline controls did not effect this relaxation within 60 min (the mean time between experimental protocols). Nifedipine (0.1 μM) effected this reduction in 2.1 ± 0.2 min (n = 4), minoxidil sulphate (1.0 μM) effected this reduction in 1.6 ± 0.3 min (n = 5).

Discussion

The findings of this direct comparative study demonstrate clearly, for the first time, that suppressive effects of progesterone on spontaneous and agonist-evoked contractions of portal vein and aorta utilise different mechanisms of action: potassium channel activation and calcium channel blockade respectively.

Suppression by progesterone of adrenoceptor-mediated contractions has been previously demonstrated on rabbit uterus and ductus deferens (Morishita 1986) and human cerebral, uterine and umbilical arteries (Eccles & Leathard 1985; Leathard et al 1992), but the mechanism of the inhibition was not explored. In the current study, inhibition of the tonic contractions of the aorta suggested inhibition of either extracellular Ca^{2+} entry or intracellular Ca^{2+} release, whereas inhibition of the phasic, but not the tonic, component of contractions of the portal vein seemed attributable to selective inhibition of extracellular Ca^{2+} entry, on which phasic contractions are more exclusively dependent than tonic (Golenhofen 1981). In agreement with this interpretation, the phasic activity of the portal vein was shown to be highly dependent on the presence of Ca^{2+} in the extracellular fluid, with complete inhibition of spontaneous activity at $0-0.5$ mM Ca^{2+}. This inhibitory action of progesterone closely resembled the effect of nifedipine in the current study, and that described for various established Ca^{2+} channel blockers (Dacquet et al 1987).

Although progesterone did not inhibit the tonic component of portal vein contractions evoked by NA, which are understood to be independent of calcium entry, it did inhibit the tonic component of Ca^{2+}_o-induced contractions, so its action was not limited to phasic components of contractions, at resting (basal E_m) conditions. Inhibition of the Ca^{2+}_o-induced aortic contractions provides further evidence of progesterone acting by inhibition of Ca^{2+}_o entry, although this does not necessarily implicate a direct action on calcium channels. The hyperpolarising effect of potassium channel opening, for example, could produce similar mechanical inhibition.

In the portal vein, the preferential suppressive action of progesterone on spontaneous and 20 mM K^+-evoked phasic components of contractions, but not on $40-80$ mM K^+-evoked contractions, suggested that this steroid could be acting as a potassium channel opener (KCO). The observed effects were comparable to those caused by minoxidil sulphate and other known KCOs, including cromakalim, lemakalim, pinacidil, S 0121, Ro 31-6930, RP 49356 and P1060 on K^+-induced contractions of the rat portal vein (Hamilton et al 1986; Newgreen et al 1989; Edwards et al 1991; present study) and support previous observations of selective suppression by progesterone on K^+-induced contractions of rat portal vein and aorta (Huyton & Leathard 1991) and human umbilical arteries (Leathard et al 1992).

The action of progesterone on K^+-induced contractions of the aorta showed a different pattern of suppressive activity with significant inhibition evident from 40 to 120 mM K^+. This inhibition of 'high' K^+-induced contractions, in contrast to that seen with the portal vein, corresponded closely to the suppression of Ca^{2+}_o-induced contractions of the aorta, which were elicited in a highly depolarising (60 mM K^+) saline. Similar observations of progesterone-induced suppression of highly depolarised tissues have been made on rat uterus (Gutierrez et al 1994), rabbit coronary arteries and rat aortae (Jiang et al 1992; Glusa et al 1997). These data strongly suggest that progesterone does not act solely as a KCO on the aorta, but that an additional or alternative inhibitory mechanism of action is in effect on this tissue, such as voltage operated Ca^{2+} channel blockade or inhibition of intracellular Ca^{2+} release. This contrasts, however, with the findings of Huyton & Leathard (1991) on rat aortae, which demonstrated a 'low K^+ only' pattern of suppression, indicative of KCO action, caused by a low (50 nM) concentration of progesterone. The hypothesis that progesterone acts predominantly as a KCO on vascular tissues at low concentrations, with an additional inhibitory mechanism at higher concentrations, in the aorta, could explain the differences between these studies. Variability in the suppressive action of progesterone has also been recorded in-vivo by Chu & Beilin (1997), who observed differences between mesenteric and renal vascular beds in changes of reactivity during pregnancy in the rat. It seems probable therefore that differences between vascular tissues in response to progesterone have functional significance.

The rapid onset of inhibition by progesterone of portal vein spontaneous contractions resembles that described by McCalden (1975), and supports Morishita's (1986) conclusion that non-genomic mechanisms are involved.

In conclusion, although electrophysiological confirmation is still required, this study has significantly extended previous work on the effects of progesterone on different vascular tissues, providing strong indirect evidence that progesterone causes inhibition of contractility by acting principally as a KCO agent on the portal vein, with a different, or additional, inhibitory mechanism on the aorta, for which the data are consistent with Ca^{2+} channel blockade.

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