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The Effects of Potassium Channel Blockers on Progesterone-Induced Suppression of Rat Portal Vein Contractility

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Abstract

The suppression of contractility of rat portal vein caused by progesterone appears to be due to the potassium (K^+) channel opening effect of this hormone. The identity of the specific K^+ channels involved has been investigated using a variety of K^+ channel blockers.

Incubation with 100 nM iberiotoxin antagonised the progesterone-induced inhibition of spontaneous and 20 mM K^+-induced phasic activity of the portal vein such that the contractions resembled those of the non-progesterone, non-iberiotoxin control tissues treated with the corresponding solvent vehicles. Incubation with barium chloride (20 and 100 μM), 4-aminopyridine (1 mM), tetraethylammonium chloride (1 mM), glibenclamide (1 μM) or apamin (1 μM) did not, however, have the same antagonistic effect.

These results suggest that progesterone's selective suppression of rat portal vein contractility is mediated by the opening of BK_{Ca} channels.

Progesterone is believed to suppress spontaneous and evoked contractions of rat portal vein by inhibition of extracellular Ca^{2+} entry via activation of K^+ channels (Mukerji et al 1995, 2000; Mukerji 1999). K^+ channels are of many different types and many agents appear to act via modulation of the open probability (P_o) of particular types of these channels (Cook 1988; Standen et al 1989; Edwards et al 1992). This paper describes the elucidation of the specific K^+ channel subtype(s) activated by progesterone by the use of K^+ channel blockers (KCBs). Relevant properties of candidate channels and the rationales for the choices of KCBs are summarised briefly below.

The spontaneous contractions of the portal vein are generated by electrical spike complexes that can 'raise' the membrane potential (E_m) up to -10 to 0 mV (Southerton et al 1988) and on which K_V inhibitors are known to have an excitatory effect (Noack et al 1992; Edwards et al 1996). It was thus thought that the opening of K_V channels could be associated to some degree with progesterone's suppressive action.

The activation of the BK_{Ca} channel requires an elevated level of intracellular Ca^{2+} with a depolarised E_m (Edwards & Weston 1995a). However, due to the high conductance of BK_{Ca} and the large number of BK_{Ca} channels present per cell, any slight increase in the P_o of this channel would have a significant effect on the basal E_m (Nelson et al 1990) and it could, therefore, contribute a functionally important component of the total K^+ conductance under resting conditions. The SK_{Ca} channel is another Ca^{2+}-sensitive K^+ channel but its activation is not voltage dependent and thus it may be open at resting E_m (Kohler et al 1996). Both of these channels could therefore be affected by progesterone.

Both the K_{ATP} and the K_{IR} channels are active at a relatively negative E_m and have been noted to be involved in setting the resting E_m level in vascular smooth muscles (Aaronson & Benham 1996). Knot et al (1996) and Quayle et al (1996) observed a vasodilator effect of 5–15 mM K^+ addition on coronary and cerebral arteries that was due to activation of K_{IR} and suggested a role for this channel in the metabolic regulation of blood flow. Several vasodilator drugs, including levcromakalim, pinacidil, nicorandil and minoxidil sulphate, have been shown to operate via the opening of K_{ATP} channels, as reviewed by Standen et al (1989), Edwards & Weston (1995b) and Nakaya & Miyoshi (1996). It was thus thought possible

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that progesterone might suppress contractility in the portal vein by activation of K_{IR} or K_{ATP} channels.

The K_A channel is active at basal conditions but is known to be rapidly inactivating (Edwards & Weston 1995a). The effect of progesterone is sustained over a period of time (> 40 min; Mukerji 1999; Mukerji et al 2000) and therefore is not consistent with the involvement of the K_A channel.

The effect of KCBs is to reduce the P_o of K^+ channels, leading to membrane depolarisation and thus excitation. If progesterone is acting as a K^+ channel opener on the rat portal vein then it should be possible to antagonise this action by the use of a corresponding KCB. In order to cover as wide a range of candidate K^+ channels as possible, an analysis of the effects of a variety of KCBs on the vasosuppressive action of progesterone was undertaken. These included several non-specific KCBs (barium, 4-aminopyridine and tetraethylammonium), which allow useful comparative analysis owing to their different relative potencies on the K^+ channel subtypes that they affect, as established from a number of studies and reviews (Cook 1988; McCarron & Halpern 1990; Nelson et al 1990; Bolton & Beech 1992; Brayden & Nelson 1992; Quayle et al 1993; Nelson & Quayle 1995; Quast et al 1995; Crossley & Opalko 1996; Edwards & Weston 1997), the results of which are summarised in Table 1. Other, highly subtype specific KCBs (glibenclamide, iberiotoxin and apamin) were selected to provide a more precise assessment of any involvement of the K^+ channels that they respectively block. A preliminary account of these studies has been presented at the British Pharmacological Society (Mukerji et al 1999).

Methods

Rats and equipment

Male Wistar albino rats, 300–400 g, were obtained from the Lancaster University animal housing facility. The rats were stunned, then killed by cervical dislocation. A section of the portal vein, ~2 cm in length, was immediately dissected out and placed in aerated Tyrode's mammalian saline.

The portal vein was suspended in a conventional isolated organ bath under an initial tension equivalent to a load of 0·5–1·0 g (maintained at 37°C). 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 mL, and were washed out by replacing the Tyrode's saline.

The amplitude of change in basal tone recorded 4 min post agonist addition was measured as the tonic component of contractions; the phasic component of contractions was measured as the mean amplitude of phasic peaks recorded over a period of 3–5 min post agonist addition.

KCBs alone

The effect of each KCB on the spontaneous activity of the portal vein was studied by the analysis of concentration–effect curves created by cumulative addition of the KCB (Figure 1). An assessment of the concentration of each KCB to be used in subsequent studies on progesterone-suppressed contractions was based on these preliminary studies and the reviews of the related literature described above.

A barium chloride (Ba^{2+}) concentration of 20 μM was used to block K_{IR} channels (McCarron & Halpern 1990; Quayle et al 1993). A Ba^{2+} concentration of 100 μM (Quayle et al 1988; Bonev & Nelson 1993; Quast et al 1995) and a 1 μM concentration of glibenclamide (Buckingham et al 1989; Standen et al 1989; Edwards et al 1992; Ibbotson et al 1993) were both used, separately, to block K_{ATP} channels. Apamin (1 μM) was used as a blocker of the SK_{Ca} channel (Bolton & Beech 1992; Kohler et al 1996). A 1 mM concentration of 4-aminopyridine (4-AP) was used to block K_V channels (Nelson & Quayle 1995; Aaronson & Benham 1996; White & Hiley 1997).

Table 1. Relative potencies of the non-specific Potassium channel blockers (KCBs) used in this study.

KCB	K^+ channels blocked			
TEA	BK_{Ca} (200 μM)	> K_{ATP} (7 mM)	> K_V (10–50 mM)	> K_{IR}
Ba^{2+}	K_{IR} (2 μM)	> K_{ATP} (100 μM)	> K_V (> 1 mM)	> BK_{Ca} (> 10 mM)
4-AP	K_V (0·2–1·1 mM)	≥K_{ATP} (0·2 mM)	> K_A > K_{IR}	> BK_{Ca} (> 5 mM)

Approximate half-inhibition constants are indicated, where known, in parentheses.

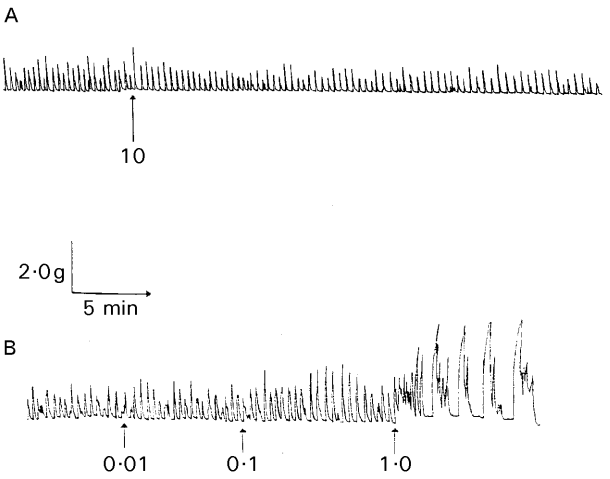

Figure 1. Changes in isometric tension of rat portal vein due to addition of progesterone (A) and Ba^{2+} (B) to Tyrode's saline. Numbers below the arrows are the accumulated μM (A) and mM (B) concentrations of the drug produced by additions at those points.

Tetraethylammonium chloride (TEA), at a concentration of 1 mM, was used to block BK_{Ca} channels (Brayden & Nelson 1992; Edwards & Weston 1997). Iberiotoxin (100 nM) was also used as a BK_{Ca} channel blocker (Brayden & Nelson 1992; Nelson & Quayle 1995; DeFarias et al 1996).

KCB interaction with progesterone

A similar protocol was followed for each KCB. The suppressive action of 10 μM progesterone incubation for 40 min on a cumulative K^+ concentration–effect curve was initially confirmed, as described by Mukerji et al (2000). The effect of a specific KCB in antagonising this effect was then assessed by 30 min incubation of the KCB (with progesterone still present) followed by a final K^+ addition trial. KCB vehicle control studies were carried out using the solvent of each KCB. Concurrent trials were also carried out with a non-progesterone, non-KCB double-vehicle control. This allowed a direct evaluation (with specific time-matched solvent control studies) of the degree of any antagonism of progesterone's suppressive effects caused by a particular KCB.

Drugs and solutions

The Tyrode's saline used had the following composition (mM): 137·0 NaCl, 5·4 KCl, 1·79 $CaCl_2$, 1·04 $MgSO_4$, 0·34 NaH_2PO_4, 11·9 $NaHCO_3$, 5·6 glucose. It was maintained at 37°C and with pH adjusted to 7·25 ± 0·05. Where K^+ (as KCl) was used as a spasmogen, the concentrations given include the K^+ (5·4 mM) present in the normal Tyrode's saline. All drugs were obtained from Sigma. TEA, 4-AP, Ba^{2+}, apamin and iberiotoxin were all dissolved in H_2O. Progesterone and glibenclamide were prepared in 70% ethanol.

Results

Potassium channel blockers

Barium chloride. Cumulative addition of Ba^{2+} (0·01–1·0 mM) caused dose-related increases of the spontaneous activity of the rat portal vein preparation, as illustrated in Figure 1. This occurred, at low concentrations of Ba^{2+}, as an increase in the amplitude of phasic activity followed, at higher concentrations, by major cyclical changes with burst pattern activity and cycles of tonic force, consisting of complex contractions interspersed by periods of quiescence. Incubation with 20 μM Ba^{2+} (n = 3) had no effect on the progesterone-induced suppression of the phasic component of contractions evoked by K^+ addition compared with the solvent control (n = 6) (Figure 2A). The tonic component of contractions was also little affected by 20 μM Ba^{2+} compared with solvent controls. Incubation with 100 μM Ba^{2+}, however, counteracted the progesterone-induced suppression of both spontaneous (5 mM K^+) and 20 mM K^+-evoked phasic components of contractions, an effect that was statistically significantly different from that of the solvent at 5 mM K^+ (96 ± 34% increase, $P < 0·05$, n = 5–6) although not at 20 mM K^+ (61 ± 39% increase, $P = 0·14$; n = 5–6) (Figure 2B). There was no significant effect of 100 μM Ba^{2+} on tonic contractions induced by K^+ addition.

Glibenclamide. The cumulative addition of glibenclamide (0·1–10 μM) caused a number of dose-related increases to the spontaneous activity of rat portal vein, resembling the pattern reported for Ba^{2+}. Incubation with a single addition of 1 μM glibenclamide caused variable alterations of the spontaneous activity of the rat portal vein preparation. Incubation with 1 μM glibenclamide (n = 15) showed a trend towards enhancement of the phasic component of contractions evoked by K^+ addition which, in progesterone-suppressed tissues, approached the values observed with the non-progesterone, non-glibenclamide control (n = 8) (Figure 2C). The changes were not, however, statistically significant when compared with the solvent vehicle (n = 7) at either 5 mM K^+ (25 ±

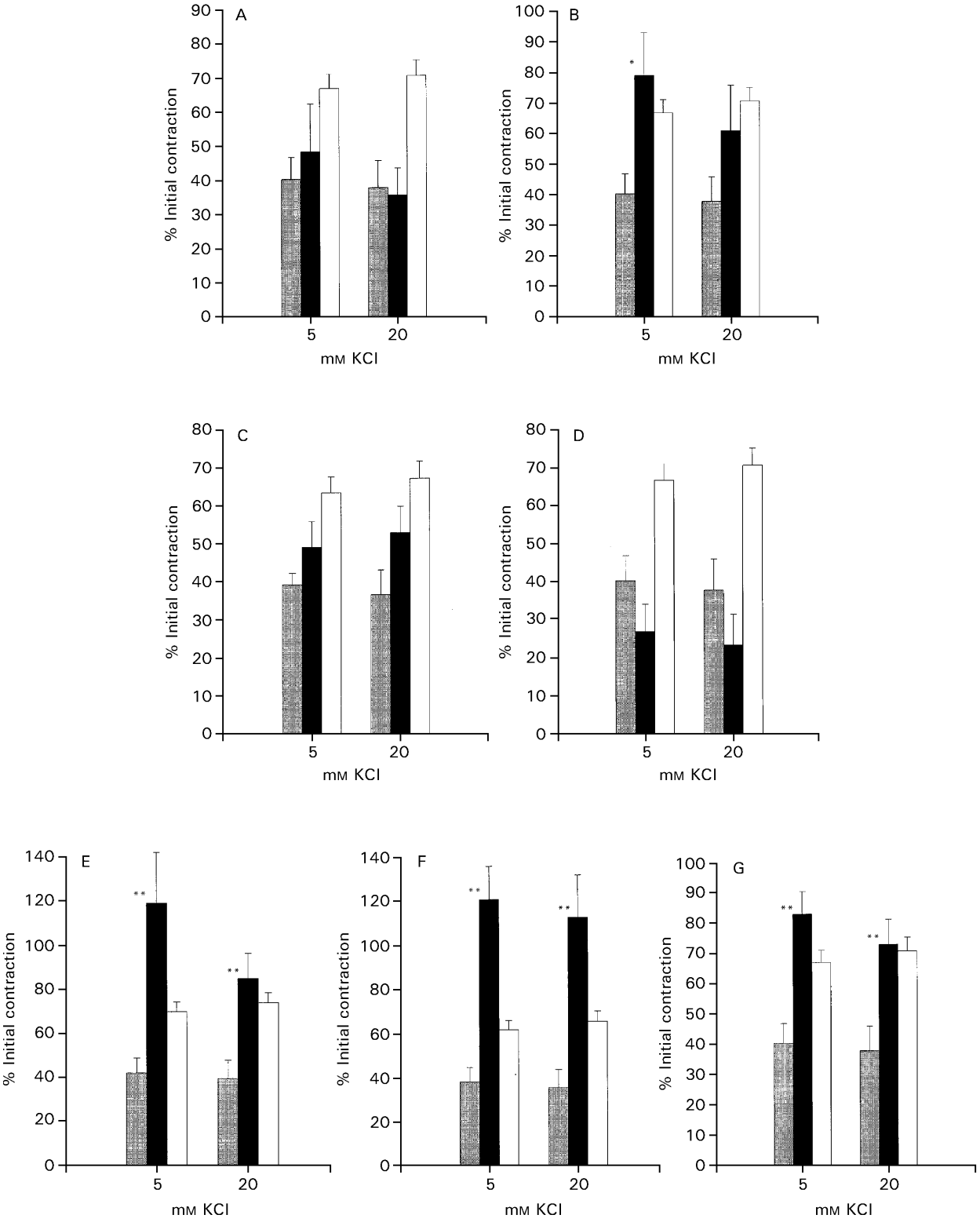

Figure 2. Histograms illustrating the effect of Potassium channel blockers (KCBs) (■) compared with their solvents (▨) on progesterone-suppressed spontaneous (5 mM K^+) and 20 mM K^+-evoked phasic contractions of rat portal vein. A. 20 μM Ba^{2+}; B. 100 μM Ba^{2+}; C. 1 μM glibenclamide; D. 1 μM apamin. E. 1 mM 4-AP; F. 1 mM TEA; G. 100 nM iberiotoxin. For comparison the non-progesterone, non-KCB control values are also included (□). On the vertical axis contractions are expressed as a percentage of the phasic contraction induced by respective K^+ concentrations prior to incubation with progesterone (10 μM). Error bars indicate the s.e.m. $^*P < 0{\cdot}05$, $^{**}P < 0{\cdot}01$ in Student's t-test between KCB and its solvent vehicle.

19% increase, $P = 0{\cdot}32$) or 20 mM K^+ ($45 \pm 36\%$ increase, $P = 0{\cdot}17$). No excitatory effects of glibenclamide were demonstrated on the tonic component of contractions evoked by K^+ addition.

Apamin. Cumulative addition of apamin (0·1–5·0 μM) caused a dose-related reduction in amplitude of spontaneous contractions of the portal vein. Incubation with 1 μM apamin ($n = 3$) appeared to potentiate the progesterone-induced suppression of the phasic component of contractions evoked by K^+ addition compared with the solvent vehicle ($n = 6$): $23 \pm 29\%$ lower at 5 mM K^+ and $28 \pm 36\%$ lower at 20 mM K^+ (Figure 2D). The tonic component of contractions was not affected by apamin.

4-Aminopyridine. Cumulative addition of 4-AP (0·1–5·0 mM) caused dose-related increases in the spontaneous activity of the portal vein. These occurred at low concentrations as an increase in the amplitude of phasic activity and at high concentrations as further increases in the amplitude of phasic activity and a development of tonic force by the preparation. Incubation with 1 mM 4-AP ($n = 8$) counteracted the progesterone-induced suppression of both spontaneous (5 mM K^+) and 20 mM K^+-evoked phasic contractions (Figure 2E). This amounted, at 5 mM K^+, to a $180 \pm 55\%$ increase ($P > 0{\cdot}01$) compared with the solvent vehicle ($n = 6$), but this was also significantly higher ($68 \pm 19\%$ greater, $P < 0{\cdot}05$) than the non-progesterone, non-4-AP control ($n = 10$). At 20 mM K^+ 4-AP caused a $115 \pm 30\%$ increase ($P > 0{\cdot}05$) that was also greater, although not significantly so, than the non-progesterone, non-4-AP control ($15 \pm 22\%$ higher, $P = 0{\cdot}45$). The tonic component of contractions was unaffected by 4-AP.

Tetraethylammonium chloride. Cumulative addition of TEA (0·1–50 mM) caused dose-related increases in the mean amplitude of phasic contractions of the spontaneously active portal vein. Incubation with 1 mM TEA ($n = 9$) counteracted the progesterone-induced suppression of both spontaneous (5 mM K^+) and 20 mM K^+-evoked phasic contractions (Figure 2F). This amounted, at 5 mM K^+, to a $200 \pm 48\%$ increase ($P > 0{\cdot}01$) compared with the solvent vehicle ($n = 6$), but this was also significantly higher ($76 \pm 20\%$ greater, $P < 0{\cdot}05$) than the non-progesterone, non-TEA control ($n = 10$). At 20 mM K^+ TEA caused a $187 \pm 39\%$ increase ($P > 0{\cdot}01$) that was also significantly higher ($59 \pm 21\%$ greater, $P > 0{\cdot}05$) than the non-progesterone, non-TEA control. The tonic component of contractions was unaffected by TEA.

Iberiotoxin. Cumulative addition of iberiotoxin (10–100 nM) caused an increase in amplitude of spontaneous activity of the portal vein. Incubation with 100 nM iberiotoxin ($n = 6$) significantly counteracted the progesterone-induced suppression of both spontaneous (5 mM K^+) and 20 mM K^+-evoked phasic contractions (Figure 2G). Compared with the solvent vehicle ($n = 6$) this amounted to a $107 \pm 18\%$ increase at 5 mM K^+ and a $82 \pm 20\%$ increase at 20 mM K^+ (both $P > 0{\cdot}01$); these values are close to those observed for the non-progesterone, non-iberiotoxin control ($n = 10$). The tonic component of contractions was unaffected by iberiotoxin compared with the solvent vehicle.

Discussion

The experiments conducted using KCBs have provided evidence consistent with the theory that progesterone causes suppression of contractility in the rat portal vein via activation of the BK_{Ca} channel, because the action of progesterone was directly counteracted by iberiotoxin but not by Ba^{2+}, apamin, glibenclamide or 4-AP.

It is notable in the analysis of changes in mechanical activity induced by pharmacological agents, such as those observed in this study, that similar patterns of effects can be produced by drugs that have different actions, in this case blockers of K_{ATP}, BK_{Ca} and K_V channels. Physiological antagonism of the effects of progesterone could have resulted from pharmacological blockade of one K^+ channel subtype that has a similar function(s) to that of the actual K^+ channel subtype activated by progesterone. For example, BK_{Ca} and K_V channels are both believed to be intrinsically involved in the repolarisation of action potentials (Edwards & Weston 1995b; Imaizumi et al 1996) and BK_{Ca} and K_{ATP} channels are both thought to have a role in the control of resting membrane potentials (Nelson et al 1990; Aaronson & Benham 1996). The identification of the BK_{Ca} channel as the sole mediator of progesterone's suppressive action on the portal vein is, therefore, somewhat speculative.

TEA (1 mM) had an excitatory action on the portal vein that counteracted the progesterone-induced suppression of spontaneous and K^+-evoked phasic contractions to the extent that the phasic activity was significantly greater than that of the non-progesterone, non-TEA control. These results are consistent with the study of Leathard et al (1990) on human intracranial arteries, which demonstrated an excitatory effect of 1 mM TEA on progesterone-suppressed contractions, evoked by

5–10 mM K^+ addition, that exceeded the amplitude of contractions of untreated control vessels. Although at this 1 mM concentration the effect of TEA is thought to be relatively selective for BK_{Ca} (Aaronson & Benham 1996; Edwards & Weston 1997), the possibility is acknowledged that there might also have been a contributory involvement of K_{ATP} and/or K_V channels, both of which have been observed to be blocked by TEA at higher concentrations (Table 1; Cook 1988; Nelson & Quayle 1995). To examine this further, the effect of the highly specific BK_{Ca} antagonist iberiotoxin was also studied. Iberiotoxin has been observed to block the opening of BK_{Ca} channels in a variety of smooth muscles, including rat portal vein (Kirkup et al 1996; Zygmunt et al 1997a). When assessed on spontaneous and K^+-evoked contractions it directly counteracted the progesterone-induced suppression. This response, unlike that seen with TEA and 4-AP, was not significantly different from its non-progesterone, non-KCB vehicle control study, strongly indicating that BK_{Ca} was the target for modulation by progesterone.

The BK_{Ca} channel is thought to be responsible for the repolarisation phase of action potentials (Imaizumi et al 1996) and as such is intrinsically linked to changes in the amplitude and frequency of spontaneous activity of the portal vein, both of which have been shown to be altered by progesterone (Mukerji et al 1995, 2000; Mukerji 1999).The current data are consistent with the observation of Jacob & White (1996) that progesterone increases the P_o of BK_{Ca} channels in porcine coronary artery cells.

The progesterone-induced suppression of portal vein phasic activity was counteracted by 100 μM Ba^{2+} and this antagonised response was not significantly different from that of the non-progesterone, non-Ba^{2+} control. Since the involvement of the K_{IR} channel appeared to have been ruled out, this indicated that the action of 100 μM Ba^{2+} as a K_{ATP} channel blocker was the predominant cause of the induced change in activity. Since Ba^{2+}, like 4-AP and TEA, is regarded as relatively non-selective, confirmatory experiments with a more specific K_{ATP} antagonist, glibenclamide, were carried out. The effect of glibenclamide on the progesterone-induced suppression of contractility of the rat portal vein showed a definite trend of excitation towards the non-progesterone, non-glibenclamide control, similar to that observed with 100 μM Ba^{2+}. Although this was not statistically significant, it seems premature, given the data observed with Ba^{2+} (100 μM), to discount a role for K_{ATP} channels in the action of progesterone unequivocally, although the large number of experiments with glibenclamide ($n = 15$), compared with other KCBs, supports the veracity of these data. Previous studies of the action of glibenclamide on rat aortae and portal veins and rabbit coronary arteries have shown no significant antagonism of progesterone's suppressive action by the K_{ATP} blocker glibenclamide (1 μM) (Huyton & Leathard 1991; Jiang et al 1992; Glusa et al 1997).

The K_V channel blocker 4-AP (1 mM) counteracted the progesterone-induced suppression of spontaneous and 20 mM K^+-induced phasic contractions of portal vein, but produced significantly greater responses than non-progesterone, non-4-AP control data, similar to that seen with TEA. There is a considerable problem of lack of specificity with the use of 4-AP, to a greater extent than with TEA and Ba^{2+}. In addition to K_V (the target channel of this experiment) the K_{ATP} channel was also likely to be blocked to a considerable degree (Beech & Bolton 1989) and, to a lesser extent, K_{IR}, K_A and BK_{Ca} blockade could possibly have contributed (Cook 1988; Nelson & Quayle 1995). Since BK_{Ca} and K_{ATP} blockade have already been shown to enhance the phasic activity of the rat portal vein, this could account for the higher than control responses effected by 4-AP. The use of more specific K_V inhibitors, such as kaliotoxin, margatoxin and terikalant (Zygmunt et al 1997b), is suggested to clarify the possible involvement of the K_V channel in the progesterone-mediated suppression of portal vein contractility.

The lack of effect of 20 μM Ba^{2+} and 0·1–5·0 μM apamin on progesterone-suppressed contractions suggested that the K_{IR} and SK_{Ca} channels are not linked to the observed inhibition and may in fact be absent from the rat portal vein.

Pharmacological analysis of K^+ channel activity is currently limited by the lack of highly selective KCBs available. This is compounded by the continuing discovery of novel channels that, although often closely associated structurally with a particular subtype of K^+ channel, may not be affected by the same subtype-specific blocker. The methodology used in this study did, however, provide a sound basis for the examination of several K^+ channels, eliminating a number of subtypes from the investigation and providing strong support for the activation of BK_{Ca} by progesterone.

An advance to this study would be an electrophysiological analysis of progesterone's action via the technique of whole-cell patch clamping of dispersed cells of the portal vein as utilised by Edwards et al (1994) and Karle et al (1998). A more direct analysis of the conductance, Ca^{2+}-dependence and voltage activation characteristics of ion channels modulated by progesterone could

thus be obtained to further elucidate the involvement of BK_{Ca} activation in the suppression of rat portal vein contractility by progesterone evident from this study.

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