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The effects of long-term infusion of salbutamol, diltiazem and nifedipine on uterine contractions in the ovariectomized, post-partum rat

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1 The sensitivity of the uterus to the inhibition of contractions by salbutamol, diltiazem or nifedipine was assessed in the ovariectomized, post-partum rat by dose-response curves following bolus intravenous (i.v.) administration. These tests were performed before (day 1), immediately after a 20 h i.v. infusion of salbutamol, diltiazem, nifedipine or appropriate control infusate (day 2) and after a further 20 h infusion of saline (day 3). In a further group of animals sensitivity to nifedipine was assessed before and after a 20 h infusion of salbutamol. Uterine contractions were monitored throughout infusions.

2 Infusion of salbutamol ($2\,\mu g\,kg^{-1}\,min^{-1}$) produced an initial marked inhibition of uterine contractions, an effect which was not maintained despite continued infusion. Contractions reappeared after 2 h of infusion and reached pre-infusion levels by 5 h. The dose-response curve to salbutamol on day 2 was shifted more than 100 fold to the right compared with that on day 1. Sensitivity of the uterus on day 3 did not differ from that on day 1.

3 Nifedipine ($25\,\mu g\,kg^{-1}\,min^{-1}$) produced sustained inhibition of uterine contractions throughout the 20 h of infusion. Sensitivity of the uterus to nifedipine could not, therefore, be tested on day 2; sensitivity on day 3 did not differ from that on day 1. In addition, there was no change in sensitivity of the uterus to nifedipine after a 20 h infusion of salbutamol.

4 Diltiazem ($200\,\mu g\,kg^{-1}\,min^{-1}$) produced a marked initial inhibition of uterine contractions, with a partial return of contractions during continued infusion in 7 out of 12 animals so that mean integral values reached 40% of those pre-infusion. The dose-response curve to diltiazem on day 2 showed a 25 fold shift to the right compared with that on day 1 in 4 out of 12 animals where the test could be performed. Sensitivity of the uterus on day 3 did not differ from that on day 1.

5 These findings suggest that marked but reversible tolerance to the inhibitory actions of salbutamol on uterine contractions occurs during long-term infusion. There was no evidence of tolerance to the uterine actions of nifedipine, but there was evidence of tolerance to diltiazem in some animals.

Introduction

Agonists at β-adrenoceptors are potent inhibitors of uterine contractions both *in vitro* and *in vivo* with a rapid onset of action (Marshall, 1970; Hollingsworth & Schnieden, 1973; Liggins & Vaughan, 1973). This direct action is the pharmacological basis for the use of compounds such as ritodrine and salbutamol in the treatment of preterm labour. However, there is controversy as to whether the use of such compounds results in a significant prolongation of gestation (Lippert, 1983; Kierse, 1984). The return of uterine contractions in a number of women treated with salbutamol for preterm labour, despite continuous infusion of salbutamol, suggested that tolerance to the drug developed with long-term exposure (Liggins & Vaughan, 1973). Repeated administration of β-adrenoceptor agonists has been found to cause desensitization to the bronchodilator actions of these compounds in asthmatics (Galant, 1983) and tolerance to other effects of β-adrenoceptor agonists has also been described (Harden, 1983). In the rat uterus a reduction in sensitivity to isoprenaline was seen after pretreatment with isoprenaline *in vitro* and *in vivo* (Johansson

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& Andersson, 1978; 1980; 1981). Recently, decreased formation of adenosine 3':5'-cyclic monophosphate (cyclic AMP) by human isolated uteri on incubation with terbutaline and a reduced effect of terbutaline on contractions of isolated uteri after treatment of the women with terbutaline have been described (Rydén *et al.*, 1982; Berg *et al.*, 1985). However, there has been no quantitative *in vivo* study of the extent of tolerance to the inhibitory action of a β-adrenoceptor agonist on uterine contractions. Such tolerance could, in part, explain their lack of effectiveness in preterm labour.

Recent studies have shown that several calcium entry blockers are able to inhibit contractions of the uterus *in vivo* after bolus intravenous injection or short-term infusion. These drugs have included nifedipine, nicardipine, diltiazem, gallopamil and verapamil in the rat (Csapo *et al.*, 1982; Abel & Hollingsworth, 1985a), nicardipine in the ewe (Golichowski *et al.*, 1985), rabbit (Lirette *et al.*, 1985) and woman (Forman *et al.*, 1981). These compounds are potent inhibitors of tension development by the rat isolated uterus as a result of inhibition of calcium influx (Granger *et al.*, 1985a,b; 1986) and their action *in vivo* is probably due to a direct action on the myometrium. Two of these compounds, diltiazem and nifedipine, showed some selectivity for inhibition of uterine contractions *in vivo* relative to their cardiovascular actions after bolus i.v. administration in the post-partum ovariectomized rat (Abel & Hollingsworth, 1985a).

The objective of the present study was to assess whether the acute effects of salbutamol, diltiazem and nifedipine observed on uterine contractions *in vivo* (Abel & Hollingsworth, 1985a) were maintained during their long-term infusion and were reversible on cessation of infusion. The experimental design adopted enabled both the rate of onset and extent of any tolerance to be assessed and whether there was any cross tolerance between nifedipine and salbutamol. Ovariectomized post-partum rats were used as uterine contractions of regular amplitude and frequency are maintained for several days in these animals (Downing & Porter, 1980; Abel & Hollingsworth, 1985a). Preliminary results have been demonstrated to the British Pharmacological Society (Abel & Hollingsworth, 1985b).

Methods

Animals

Post-partum Sprague-Dawley rats, 200–300 g, were supplied by the Animal Unit, Manchester University. Bilateral ovariectomy, jugular vein cannulation and uterine balloon insertion were carried out between 10.00 and 14.00 h within 48 h of delivery using techniques described previously (Abel & Hollingsworth, 1985a). The balloon cannula was connected to an overhead swivel device which allowed free movement of the animal within the cage.

Assessment of drug effects

Animals were allowed 24 h to recover from surgery. Intra-uterine pressure was recorded continuously on a 2-channel polygraph (Grass Instruments, Quincy, Mass., U.S.A.) and quantified as the integral of intra-uterine pressure above basal pressure using a 7P10B integrator pre-amplifier. A rise in intra-uterine pressure was assumed to be due to a contraction of the uterus.

The sensitivity of the uterus to the inhibition of contractions was assessed on day 1, before commencement of infusion, by bolus i.v. injection of the test drug; one drug per animal. The same drug or solvent in controls (sterile saline for salbutamol and diltiazem, nifedipine vehicle for nifedipine) was then infused for 20 h. The drugs were infused at approximately equieffective dose rates in a volume input of $0.4\,ml\,h^{-1}$. These dose rates, determined by preliminary experiments, were those which caused a peak inhibition of uterine contractions of between 75 and 95% within the first 1 h of infusion. These rates were: salbutamol $2\,\mu g\,kg^{-1}\,min^{-1}$, diltiazem $200\,\mu g\,kg^{-1}\,min^{-1}$ and nifedipine $25\,\mu g\,kg^{-1}\,min^{-1}$. Sensitivity of the uterus was retested, where possible, at the end of the infusion period on day 2. Sterile saline was then infused in a volume input of $0.4\,ml\,h^{-1}$ from day 2 in all animals for a further 20 h. A third sensitivity test was carried out at the termination of saline infusion on day 3. In a further group of animals sensitivity of the uterus to nifedipine was assessed on day 1 and again after a 20 h infusion of salbutamol ($2\,\mu g\,kg^{-1}\,min^{-1}$).

The response to bolus i.v. injections of the drugs was assessed by the method of Abel & Hollingsworth (1985a). Integrals of intra-uterine pressure were counted for the 10 min interval immediately after drug injection and expressed as a percentage of the integrals in the 10 min interval immediately before injection. This time period was chosen as encompassing the peak effect of all three drugs. The effects of the drugs (or solvent) during the infusion periods were assessed by measurement of the integral of intra-uterine pressure, expressed in absolute units of mmHg min, in 5 min intervals for the first 5 h of infusion and then in 1 h intervals. The integral values in the 1 h intervals were divided by 12 so that all data was expressed as mmHg min per 5 min.

Drugs

The following drugs were used: salbutamol sulphate (Glaxo), nifedipine (Bayer) and (+)-*cis* diltiazem HCl

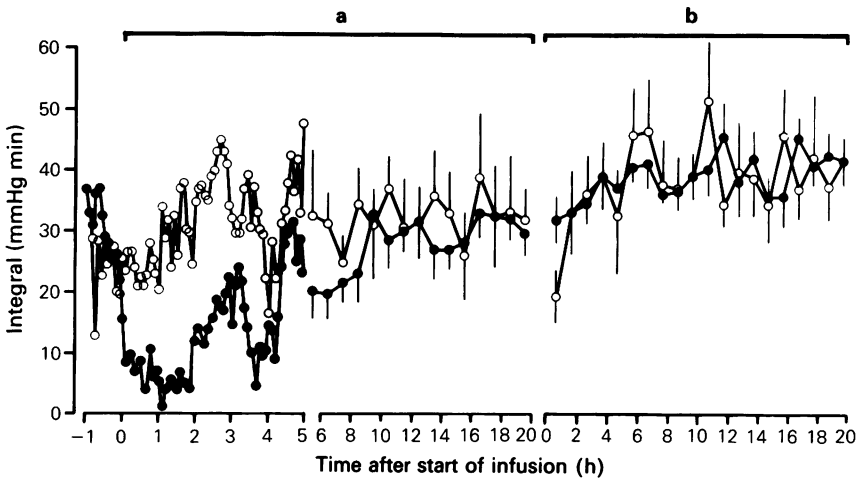

Figure 1 Uterine contractions in post-partum ovariectomized rats before infusion, during the first infusion (a) with salbutamol ($2\,\mu g\,kg^{-1}\,min^{-1}$, ●) or saline ($0.4\,ml\,h^{-1}$, ○) for 20 h followed by a second infusion (b) with saline in test (●) or control (○) animals for a further 20 h. Data are expressed as the integral (mmHg min) measured in 5 min intervals before infusion and up to 5 h after the start of the first infusion and then in 1 h intervals. Integrals were corrected to 5 min intervals. Points are the means with vertical lines indicating s.e.mean, the latter are not shown for the first 5 h for clarity. Salbutamol, $n = 8$; controls, $n = 5$.

(Synthelabo). Salbutamol and diltiazem were administered in sterile saline. Nifedipine was administered in polyethylene glycol 400:ethanol:water (15:15:50; v:v:v). Nifedipine solutions were protected from light and all experiments with this compound were carried out under sodium lighting. Doses are expressed as the base.

Results

Salbutamol infused animals

In 5 control animals the integral of uterine contractions remained relatively constant during the two 20 h periods of saline infusion (Figure 1). Infusion of salbutamol ($2\,\mu g\,kg^{-1}\,min^{-1}$) in 8 animals produced a rapid inhibition of uterine contractions to integral values of about 20% of pre-infusion levels within 5 to 10 min and were maintained at this level for 2 h (Figure 1). Reappearance of significant uterine contractions was seen at 2 h with a return to pre-infusion levels by 5 h despite continued infusion of salbutamol. Uterine contractions were of similar magnitude in the test and control groups during the second, saline infusion period.

In the 5 control animals there were no differences in the positions of the salbutamol dose-response curves following bolus administration between days 1, 2 or 3 (Figure 2a). In the 8 animals infused with salbutamol the dose-response curve to salbutamol on day 2 was shifted more than 100 fold to the right compared with day 1 (Figure 2b). The position of the dose-response curve to salbutamol in the test animals on day 3 did not differ from that on day 1.

Diltiazem infused animals

In 6 control animals the integral of uterine contractions remained constant during the two 20 h periods of saline infusion (Figure 3). Infusion of diltiazem ($200\,\mu g\,kg^{-1}\,min^{-1}$) in 12 animals induced a rapid inhibition of uterine contractions to integral values less than 10% of those pre-infusion within 10 to 20 min (Figure 3). In 7 out of the 12 test animals there was a partial return of uterine contractions during the period of diltiazem infusion so that mean integral values reached approximately 40% of those pre-infusion. These contractions differed from those seen pre-infusion, being of lower amplitude and higher frequency. In the 12 diltiazem infused animals, during the second period of infusion with saline, there was a return of uterine contractions in all animals at an approximately linear rate over the first 10 h. The pattern of uterine contractions at this time was again of short duration and high frequency with amplitude increasing with time. The integral of uterine contractions in the test animals in this second, saline infusion period reached values higher than pre-infusion values in the same animals and greater than the integrals at the corresponding time in control animals.

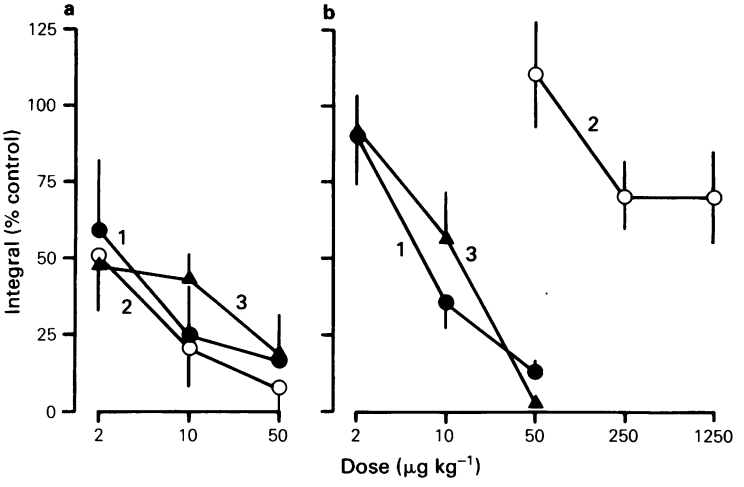

Figure 2 Dose-response curves for the inhibition of uterine contractions by bolus i.v. injection of salbutamol in controls (a) or in salbutamol infused rats (b) before infusion (day 1, ●), after the first 20 h period of infusion of saline or salbutamol (day 2, ○) and after a further 20 h period of infusion of saline (day 3, ▲). The ordinate scale is the integral of intra-uterine pressure in the 10 min period after drug injection as a % of that in the 10 min period before injection. Points are the means with vertical lines indicating s.e.mean, salbutamol, $n = 8$; controls $n = 5$.

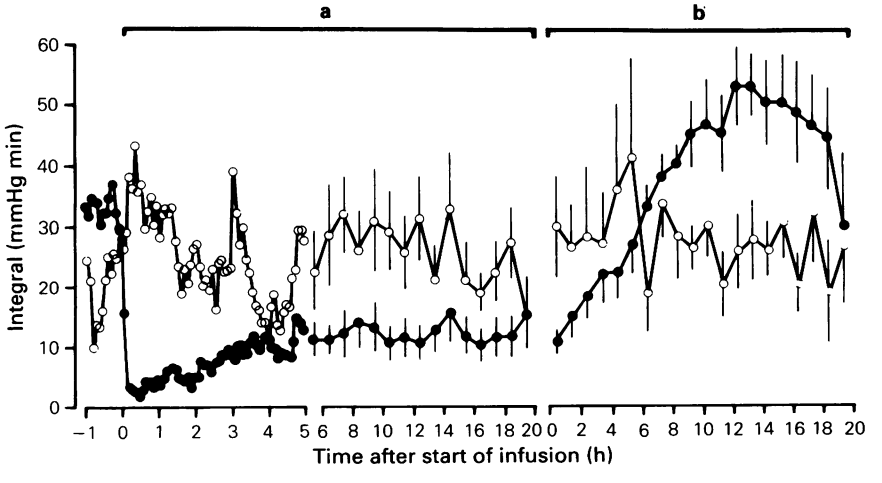

Figure 3 Uterine contractions in post-partum ovariectomized rats before infusion, during the first infusion (a) with diltiazem (200 μg kg^{-1} min^{-1}, ●) or saline (0.4 ml h^{-1}, ○) for 20 h followed by a second infusion (b) with saline in test (●) or control (○) animals for a further 20 h. Data are expressed as the integral (mmHg min) measured in 5 min intervals before infusion and up to 5 h after the start of the first infusion and then in 1 h intervals. Integrals were corrected to 5 min intervals. Points are the means with vertical lines indicating s.e.means, the latter are not shown for the first 5 h for clarity. Diltiazem, $n = 12$; controls, $n = 6$.

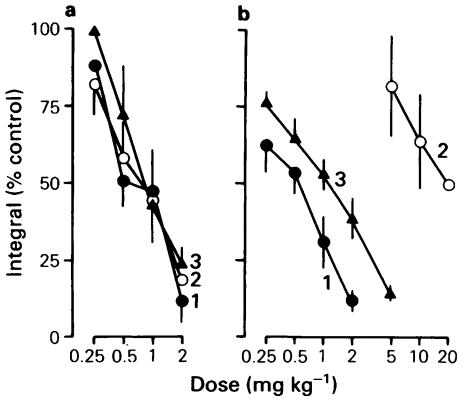

Figure 4 Dose-response curves for the inhibition of uterine contractions by bolus i.v. injection of diltiazem in controls (a) or in diltiazem infused rats (b) before infusion (day 1, ●), after the first 20 h period of infusion of diltiazem or saline (day 2, ○) and after a further 20 h period of infusion of saline (day 3, ▲). The ordinate scale is the integral of intra-uterine pressure in the 10 min period after drug injection as a % of that in the 10 min period before injection. Points are the means with vertical lines indicating s.e.mean, diltiazem $n = 12$, controls $n = 6$.

In the 6 control animals there were no differences in the positions of the diltiazem dose-response curves following bolus administration between days 1, 2 or 3 (Figure 4a). In 4 of the 12 animals infused with diltiazem there was sufficient return of regular uterine contractions to allow a sensitivity test to be carried out on day 2. In these animals there was a 25 fold rightward shift of the dose-response curve to diltiazem on day 2 compared with that on day 1 (Figure 4b). It was not possible to test the sensitivity of the uterus to bolus doses of diltiazem in the remaining 8 animals on day 2 due to the low level or irregular pattern of contractions. The position of the dose-response curve to diltiazem in the 12 animals on day 3 did not differ from that on day 1.

Nifedipine-infused animals

In 5 control animals the integral of uterine contractions remained constant during the 20 h periods of nifedipine vehicle and saline infusions (Figure 5). Infusion of nifedipine (25 $\mu g\ kg^{-1}\ min^{-1}$) in 7 animals produced a rapid inhibition of uterine contractions to integral values less than 10% of those pre-infusion within 10 to 15 min (Figure 5). This inhibition was maintained throughout most of the infusion period, although there was a slight increase in contractions

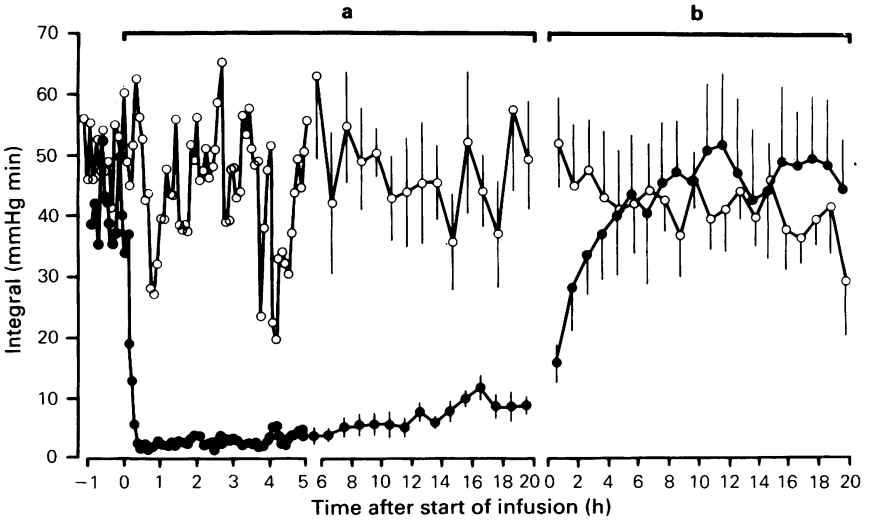

Figure 5 Uterine contractions in post-partum ovariectomized rats before infusion, during the first infusion (a) with nifedipine (25 $\mu g^{-1}\ kg^{-1}\ min^{-1}$, ●) or vehicle (0.4 ml h^{-1}, ○) for 20 h followed by a second infusion (b) with saline in test (●) or control (○) animals for a further 20 h. Data are expressed as the integral (mmHg min) measured in 5 min intervals before infusion and up to 5 h after the start of the first infusion and then in 1 h intervals. Integrals were corrected to 5 min intervals. Points are the means with vertical lines indicating s.e.mean, the latter are not shown for the first 5 h for clarity. Nifedipine, $n = 7$; controls, $n = 5$.

between 15 and 20 h with integral values approaching 20% of those pre-infusion. In the test animals, during the second period of infusion with saline, there was a return of uterine contractions to pre-infusion values by 6 h. During this time the contractions were of shorter duration and higher frequency than those observed pre-infusion and resembled those seen in the diltiazem infused animals.

In the 5 control animals there were no differences in the positions of the nifedipine dose-response curves following bolus administration between days 1, 2 or 3 (Figure 6a). The sensitivity of the uterus to bolus doses of nifedipine could not be tested on day 2 in nifedipine infused animals because of the low level of uterine contractions. Sensitivity on day 3 did not differ from that seen on day 1 (Figure 6b).

In a further group of 5 rats, sensitivity of the uterus to bolus doses of nifedipine was assessed before (day 1) and after (day 2) the animals were infused with salbutamol ($2\,\mu g\,kg^{-1}\,min^{-1}$) for 20 h. Salbutamol infusion produced a non-sustained inhibition of uterine contractions with a similar time course to that shown in Figure 1 (data not shown). The sensitivity of the uterus to bolus doses of nifedipine on day 2 was not different from that on day 1 (Figure 6c). A second period of infusion with saline was not performed in these animals.

Discussion

A rapid inhibition of uterine contractions was observed after commencement of salbutamol infusion in the current experiments in line with previous studies demonstrating similar effects following bolus i.v. injection in the pregnant (Hollingsworth & Schnieden, 1973) and post-partum ovariectomized rat (Abel & Hollingsworth, 1985a). However, this inhibition was not maintained despite continued infusion, uterine contractions reappeared after 2 h and reached pre-infusion values by 5 h. This observation strongly suggests that tolerance to the inhibitory action of salbutamol on uterine contractions had occurred. This conclusion is supported by the very marked (>100 fold) decrease in sensitivity of the uterus to bolus i.v. injection of salbutamol after infusion compared with pre-infusion. There have been other observations of a reduction in the inhibitory effects of a number of β-adrenoceptor agonists on the uterus during their prolonged administration (see Introduction). However, tolerance has not been quantified previously and tolerance of this magnitude has not been described. Such tolerance is likely to have a pharmacodynamic rather than a pharmacokinetic basis as similar observations have been made on several cell types both *in vivo* and *in vitro*. These tissues have

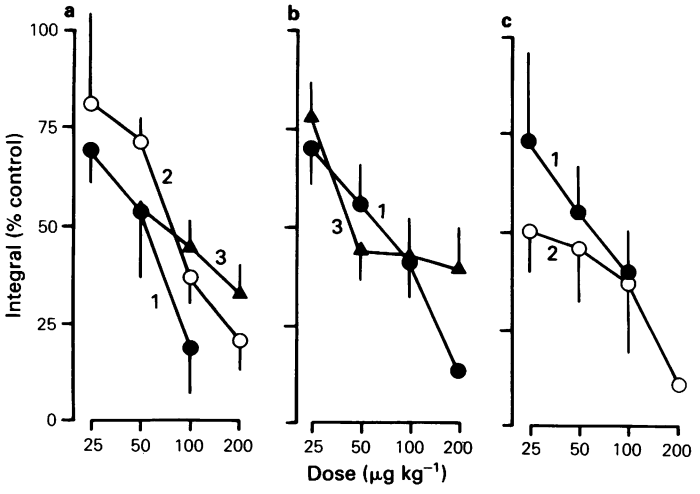

Figure 6 Dose-response curves for the inhibition of uterine contractions by bolus i.v. injection of nifedipine in controls (a), in nifedipine infused rats (b) or in salbutamol infused rats (c), before infusion (day 1, ●), after the first 20 h period of infusion of nifedipine vehicle, nifedipine or salbutamol (day 2, ○) and after a further 20 h period of infusion of saline (day 3, ▲). The ordinate scale is the integral of intra-uterine pressure in the 10 min period after drug injection as a % of that in the 10 min period before injection. Points are the means with vertical lines indicating s.e.mean, nifedipine, $n = 7$; controls, $n = 5$; salbutamol, $n = 5$.

included smooth muscles other than uterus, leucocytes, glioma cells, cardiovascular tissues and adipocytes (Wager *et al.*, 1981; Frederich *et al.*, 1983; Harden, 1983; Berg *et al.*, 1984; Stiles *et al.*, 1984). Some of the biochemical changes associated with tolerance to β-adrenoceptor agonists such as decreased production of cyclic AMP and decrease in number of binding sites for β-adrenoceptor agonists on repeated challenge with the agonist have been described in the myometrium of rat and human uterus (Johansson & Andersson, 1980; Berg *et al.*, 1985). Infusion of salbutamol into the ovariectomized, oestrogen-treated pregnant rat failed to prevent preterm delivery and this is likely to be a consequence of tolerance to the action of salbutamol on the uterus (Abel & Hollingsworth, 1986). Tolerance to salbutamol in the present study was reversible as the sensitivity of the uterus on day 3 to bolus salbutamol, after a 20 h period of saline infusion, was not different from that on day 1.

In contrast to salbutamol, the two calcium entry blockers, nifedipine and diltiazem, were capable of producing prolonged inhibition of uterine contractions in the post-partum ovariectomized rat. Nifedipine and diltiazem share the ability to inhibit calcium influx into rat myometrium *in vitro* and reduce contractions of the rat isolated uterus (Granger *et al.*, 1985a,b; 1986). This is likely to be the pharmacological basis of their inhibition of contractions of the rat uterus *in vivo* after bolus i.v. injection (Abel & Hollingsworth, 1985a) and i.v. infusion (present study).

Previous studies have demonstrated the ability of nifedipine, or the related compound nicardipine, to inhibit uterine contractions *in vivo* after bolus i.v. injection, single oral dose or short-term infusion (see Introduction). We have now demonstrated that sustained inhibition of uterine contractions can be achieved with nifedipine during prolonged infusion (20 h), an effect which was reversible on cessation of infusion. There was no evidence of tolerance to this action of nifedipine. The maintained inhibition of uterine contractions would explain the ability of this compound to extend gestation in ovariectomized, oestrogen-treated pregnant rats (Csapo *et al.*, 1982; Abel & Hollingsworth, 1986).

In rats infused with salbutamol, where tolerance to salbutamol developed, there was no change in sensitivity of the uterus to bolus doses of nifedipine. Therefore, there was no cross tolerance between nifedipine and salbutamol. This observation strongly supports the idea that nifedipine and salbutamol inhibit uterine contractions by different mechanisms.

The present study has shown that infusion of diltiazem produced sustained inhibition of uterine contractions in the majority of rats. This extends a previous finding that diltiazem can produce short-term inhibition of uterine contractions after bolus i.v. injection (Abel & Hollingsworth, 1985a). The current observation would explain the ability of this compound to prolong parturition in rats when administered orally at term (Hahn *et al.*, 1984) and to extend gestation when infused into ovariectomized, oestrogen-treated pregnant rats (Abel & Hollingsworth, 1986).

In approximately half of the animals infused with diltiazem there was a partial return of uterine contractions during the later part of the infusion period accompanied, in those tested, by a marked decrease in sensitivity of the uterus to bolus doses of diltiazem on day 2 compared with day 1. The simplest explanation for these findings is that there was tolerance to diltiazem and, as far as we are aware, this is the first report of such an observation. It is likely that the tolerance has a pharmacodynamic rather than a pharmacokinetic basis due to the marked change in sensitivity observed.

Sub-classes of calcium entry blockers have been proposed on the basis of functional criteria (Spedding, 1982; Granger *et al.*, 1985a) and ligand-binding data (Glossmann *et al.*, 1982). Nifedipine and diltiazem are suggested to be members of different sub-classes. The observation of tolerance to diltiazem but not to nifedipine provides further support for the idea that diltiazem and nifedipine exert their actions via different sites.

In summary, although salbutamol, nifedipine and diltiazem can inhibit uterine contractions acutely *in vivo* (Abel & Hollingsworth, 1985a), this action cannot necessarily be extrapolated to predict a maintained inhibition of uterine contractions during prolonged administration. Salbutamol was unable to produce sustained inhibition of uterine contractions in the post-partum ovariectomized rat due to the development of tolerance. Nifedipine and diltiazem induced inhibition of uterine contractions throughout the period of infusion, but there was evidence of tolerance to diltiazem in some animals.

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