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Nifedipine kinetics in the rat and relationship between its serum concentrations and uterine and cardiovascular effects

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1 The kinetics of nifedipine and the relationship between its serum concentration and uterine and cardiovascular effects were investigated in 3 groups of animals. These were ovariectomized (ovx) anaesthetized non-pregnant rats following bolus i.v. injection (400 $\mu g\,kg^{-1}$) and during 300 min infusion (10 $\mu g\,kg^{-1}\,min^{-1}$) and ovx, progesterone-treated late pregnant rats during infusion. Also, the kinetics were determined in ovary-intact late pregnant rats following bolus i.v. injection (400 $\mu g\,kg^{-1}$).

2 Measurement of serum nifedipine concentrations after bolus i.v. injection in ovx non-pregnant rats showed a biexponential decay with time from which the following parameters were calculated: $V_\beta = 300 \pm 30\,ml\,kg^{-1}$; rate constants $k_{12} = 0.51 \pm 0.18\,min^{-1}$; $k_{21} = 0.07 \pm 0.02\,min^{-1}$; $k_{el} = 0.10 \pm 0.05\,min^{-1}$; elimination clearance $= 2.4 \pm 0.2\,(ml\,min^{-1})\,kg^{-1}$; $t_{1/2\alpha} = 2.5 \pm 1.0$ min; $t_{1/2\beta} = 102 \pm 15$ min. In intact pregnant rats, a biexponential decay of serum nifedipine concentrations with time was also observed after bolus i.v. administration with similar parameters to non-pregnant animals. These kinetic parameters, used to calculate serum nifedipine concentrations obtained during infusion, predicted values similar to experimental values for 180 min, but thereafter slightly underestimated experimental values.

3 Immediate reductions in uterine contractions, mean blood pressure and heart rate were observed following bolus i.v. injection of nifedipine to ovx non-pregnant rats, with returns towards control values as serum nifedipine concentrations declined. IC_{15} values (15% change from baseline), calculated from log_{10} serum concentration-response curves, of $0.3 \pm 0.05\,\mu g\,ml^{-1}$ for inhibition of uterine contractions, $0.8 \pm 0.3\,\mu g\,ml^{-1}$ for depression of blood pressure and $3.8 \pm 1.0\,\mu g\,ml^{-1}$ for reduction in heart rate were obtained.

4 In ovx non-pregnant rats, nifedipine infusion produced a maximum reduction in integral of uterine contractions of 70% by 120 min and a maximum reduction of 15% in heart rate. Mean blood pressure was not significantly different from vehicle-treated rats. IC_{15} values were $0.7 \pm 0.1\,\mu g\,ml^{-1}$ and $2.8 \pm 0.6\,\mu g\,ml^{-1}$ for inhibition of uterine contractions and heart rate respectively.

5 In ovx, progesterone-treated late pregnant rats, nifedipine infusion produced similar serum concentrations to those of non-pregnant rats but completely abolished uterine contractions by 70 min. Maximum reductions of 30% in heart rate and blood pressure were observed. IC_{15} values were $0.5 \pm 0.1\,\mu g\,ml^{-1}$ for uterine contractions, $0.9 \pm 0.3\,\mu g\,ml^{-1}$ for blood pressure and $1.2 \pm 0.3\,\mu g\,ml^{-1}$ for heart rate.

6 The findings suggest that the kinetics of nifedipine are similar in pregnant and non-pregnant rats and support the idea that the drug exerts a slight selectivity for uterine inhibition relative to cardiovascular effects. The uterus of the late pregnant rat appears to be more sensitive to nifedipine than that of the non-pregnant animal.

Introduction

The calcium entry blockers are a group of structurally diverse compounds, a number of which have been shown to be potent inhibitors of tension development by uterine smooth muscle *in vitro* (Edwards *et al.*, 1986; Granger *et al.*, 1985; 1986) and *in vivo* (Forman *et al.*, 1982; Golichowski *et al.*, 1985; Abel & Hollingsworth, 1985; 1986a; Downing *et al.*, 1987; Hollingsworth & Downing, 1988). Further-

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more, (+)-*cis* diltiazem and nifedipine were shown to be effective in preventing preterm delivery when administered by infusion to ovariectomized (ovx), oestrogen-treated pregnant rats (Abel & Hollingsworth, 1986b). In these *in vivo* studies, nifedipine was found to exert slight selectivity for the uterus relative to cardiovascular effects. Selectivity of nifedipine on uterine tissue compared to vascular and ventricular tissue was also observed *in vitro* (Granger *et al.*, 1985).

The physiological changes accompanying pregnancy may markedly affect both pharmacokinetics and pharmacodynamics of drugs (Krauer *et al.*, 1984). We recently demonstrated that pregnant rats exhibited a significantly increased elimination clearance of diltiazem compared to non-pregnant rats (Downing *et al.*, 1987), such that lower serum diltiazem concentrations were achieved in pregnant rats than in non-pregnant animals by the same infusion rate. It is not known if similar changes in kinetic parameters as a result of pregnancy are observed with other calcium entry blockers. Additionally, it is not known whether the action and potency of nifedipine on the myometrium is affected by hormonal changes associated with pregnancy. Therefore, the kinetics and the relationship between serum concentrations of nifedipine and uterine and cardiovascular effects have been studied in detail in ovx non-pregnant and late pregnant rats following bolus i.v. injection and infusion. Preliminary data have been published (Downing & Hollingsworth, 1987).

Methods

Non-pregnant (200–250 g) and timed pregnant rats (day 18–21 of pregnancy) were supplied by the Animal Unit, University of Manchester. Day of mating was designated day 1 of pregnancy.

Bolus dose

Non-pregnant rats The rats were anaesthetized with tribromoethanol (240 mg kg^{-1}) i.p., subjected to bilateral ovariectomy via dorsal laparotomy and equipped with a small latex pressure recording balloon inserted into one uterine horn as described previously (Downing *et al.*, 1987). The rats were reanaesthetized 24 to 48 h later with tribromoethanol and carotid arterial and jugular venous cannulae inserted (Downing *et al.*, 1987). The rats received an i.v. bolus injection of either nifedipine (400 μg kg^{-1}, $n = 9$) or 0.1 ml vehicle (polyethylene glycol : ethanol : saline; 3 : 3 : 10; $n = 10$). Blood samples (0.2 ml) were taken at the following times after injection of nifedipine: 1, 2, 5, 10, 20, 40, 60, 80, 100, 120, 150 and 180 min. The blood removed was replaced with an equal volume of normal saline containing 20 u heparin per ml. Uterine contractions, blood pressure and heart rate were monitored for 30 min before and 180 min after the injection of either nifedipine or vehicle as described by Downing *et al.* (1987).

Pregnant rats As only kinetic data were derived from these animals, they were not subjected to prior ovariectomy or balloon implantation. They were anaesthetized with tribromoethanol and carotid arterial and jugular venous cannulae were inserted. Blood samples were taken after i.v. bolus injection of nifedipine (400 $\mu g\,kg^{-1}$) at the times indicated for non-pregnant animals.

Infusion

Non-pregnant rats The rats were anaesthetized with tribromoethanol, bilaterally ovariectomized and a uterine balloon inserted. The rats were reanaesthetized 24–48 h later with tribromoethanol and carotid arterial and jugular vein cannulae placed. The rats received an i.v. infusion via the left jugular cannula of either nifedipine (10 $\mu g\,kg^{-1}\,min^{-1}$, $n = 10$) or vehicle (polyethylene glycol : ethanol : saline, 3 : 3 : 10; 0.0069 ml min^{-1}, $n = 10$) for 300 min. Blood samples (0.2 ml) were taken from the right jugular cannula at the following times during infusion: 5, 10, 20, 30, 60, 120, 180, 240 and 300 min. The blood removed was replaced with saline containing heparin. Uterine contractions, blood pressure and heart rate were monitored for 30 min before and 300 min during the infusion of either nifedipine or vehicle.

Pregnant rats These animals, in addition to ovariectomy and balloon implantation, also received a s.c. silastic implant (5 cm long × i.d. 0.058 in × o.d. 0.077 in, Dow Corning, Midland, MI, U.S.A.) containing approximately 50 mg crystalline progesterone. These progesterone implants have been shown previously to produce serum progesterone concentrations similar to those of late pregnant rats and to maintain pregnancy for up to 12 days (Downing & Sherwood, 1985). The rats were reanaesthetized 24 to 48 h later with tribromoethanol and carotid arterial and jugular vein cannulae placed. The rats received an i.v. infusion via the left jugular cannula of either nifedipine (10 $\mu g\,kg^{-1}\,min^{-1}$, $n = 5$) or vehicle (polyethylene glycol : ethanol : saline, 3 : 3 : 10, 0.0069 ml min^{-1}, $n = 5$) for 300 min. Blood samples (0.2 ml) were taken from the right jugular cannula at

the following times during infusion: 5, 10, 20, 30, 60, 120, 180, 240 and 300 min. The blood removed was replaced with saline containing heparin. Uterine contractions, blood pressure and heart rate were monitored for 30 min before and 300 min during the infusion of either nifedipine or vehicle.

All animals were wrapped in a cotton wool blanket to maintain body temperature at 37°C. They were killed without recovery from anaesthesia.

High performance liquid chromatography

All blood samples were placed immediately on ice until centrifuged at 15,600 *g* (MSE microcentaur centrifuge) for 10 min and the serum collected. Heparin was used to prevent clotting in the jugular vein between blood sample collections. However, the dose was too small to produce a systemic anticoagulant action and thus drug concentrations were measured in serum, not plasma. The serum samples were stored at −20°C until assayed for nifedipine by h.p.l.c. by the method of Waller *et al.* (1984).

The h.p.l.c. system was as described previously (Downing *et al.*, 1987). The columns used were a 4.6 mm × 150 mm Spherisorb 3 ODS 2 analytical column with a Co Pell ODS guard column (Technicol, Stockport, Cheshire); h.p.l.c. grade solvents (Rathburn Ltd., Walkerburn, Peebleshire) and Analar grade reagents were used throughout. Sensitivity, accuracy, recovery and inter- and intra-assay variation were determined as described previously (Downing *et al.*, 1987).

Pharmacokinetic analysis

Initial analysis of data derived from bolus dose assumed a biexponential decay of concentrations of nifedipine against time. Using a Nelder and Mead non-linear curve-fitting optimization computer programme (Box *et al.*, 1969) the intercept and slope of the initial exponential phase (A and α) and of the terminal exponential phase (B and β) (Notari, 1980) were calculated using data from individual rats. From these intercepts and slopes, the following kinetic parameters were calculated: distribution $t_{1/2}$ ($t_{1/2\alpha}$), elimination $t_{1/2}$ ($t_{1/2\beta}$), rate constants (k_{12}, k_{21}, k_{el}), area under the curve (AUC) = (A/α) + (B/β), elimination clearance (CL_{el}) = dose/AUC, volume of distribution (V_β) = CL_{el}/β. Values of AUC were also checked by calculation by the trapezoidal method (Notari, 1980). No appreciable differences in the values of AUC calculated by the two methods were observed. Kinetic parameters were than used to predict values for serum nifedipine concentration obtained during infusion for a two-compartment system (Notari, 1980).

Concentration-effect relationship

The percentage changes in integral of uterine contractions compared to mean pretreatment values were measured between 0 and 5 min and encompassing every subsequent time of blood sampling after bolus injection and during infusion for each animal. The percentage changes in blood pressure and heart rate compared to mean pretreatment values were determined at 1 min (bolus dose) and every subsequent time of blood sampling. Thus, for each animal, the serum concentration of nifedipine and the change in biological parameters were determined at the same times. The mean percentage reductions in biological parameters at each time of blood sampling in vehicle-treated non-pregnant and pregnant control rats were subtracted from the reduction observed in nifedipine-treated animals prior to curve-fitting to correct for the effects of vehicle, prolonged anaesthesia and repeated blood sampling. Curves were fitted to the corrected concentration-effect data from individual animals by polynomial curve fitting (Sokal & Rohlf, 1969). Since 100% inhibition was not achieved in all parameters, the concentration at which 15% reduction (absolute value) in biological parameter was determined, where possible, for each animal. For the purposes of illustration, curves shown in figures are fitted from the mean data of all animals in each experimental group.

Statistics

Kinetic data derived from individual non-pregnant and pregnant rats were subjected to Bartlett's test for homogeneity, then compared by Student's *t* test (2-tailed) (Sokal & Rohlf, 1969). Means of frequency and integral of uterine contraction, blood pressure and heart rate were compared between treatment groups by repeated measures analysis of variance (SPSS, 1981) followed by Student's *t*-test.

Drugs

Nifedipine and nitrendipine were supplied by Bayer U.K. Limited, Newbury, Berkshire. All procedures involving nifedipine were performed under yellow light or reduced light to prevent photodecomposition of the drug. Polyethylene glycol (mol.wt. = 400), heparin and progesterone were obtained from Sigma Chemical Co. (Poole, Dorset), tribromoethanol was obtained from Fluka Chemicals (Glossop, Derbyshire).

Table 1 Kinetic parameters for nifedipine derived from bolus dose (400 $\mu g\,kg^{-1}$) and infusion (10 $\mu g\,kg^{-1}\,min^{-1}$)

Parameter	Non-pregnant		Pregnant	
	Bolus	*Infusion*	*Bolus*	*Infusion*
Body weight (kg)	0.22 ± 0.004	0.23 ± 0.006	0.32 ± 0.008	0.35 ± 0.016
V_β ($ml\,kg^{-1}$)	300 ± 30	300 ± 40	400 ± 70	200 ± 50
k_{12} (min^{-1})	0.51 ± 0.18		0.56 ± 0.13	
k_{21} (min^{-1})	0.07 ± 0.02		0.10 ± 0.02	
k_{el} (min^{-1})	0.10 ± 0.05		0.12 ± 0.07	
CL_{el} ($ml\,min^{-1}$)kg^{-1}	2.4 ± 0.2	0.9 ± 0.2	2.7 ± 0.5	1.1 ± 0.4
$t_{1/2\alpha}$ (min)	2.5 ± 1.0		1.4 ± 0.3	
$t_{1/2\beta}$ (min)	102 ± 15		141 ± 30	

Values are means ± s.e.mean (non-pregnant, bolus $n = 9$, infusion $n = 10$; pregnant, bolus $n = 10$, infusion $n = 5$). V_β = volume of distribution; k_{12}, k_{21} and k_{el} = rate constants; CL_{el} = elimination clearance; $t_{1/2\alpha}$ and $t_{1/2\beta}$ = distribution and elimination half-times respectively.

Results

Kinetic studies

H.p.l.c. The assay showed a high recovery of nifedipine and nitrendipine from serum (93 and 100% respectively) and was precise (intra-assay CV, 8% at 25 ng, 2% at 1000 ng; inter-assay CV, 10% at 25 ng, 4% at 1000 ng). The method was also sensitive (minimum measurable concentration was 10 $ng\,ml^{-1}$) and showed no bias (accuracy—mean deviation from expected value <9%).

Bolus dose h.p.l.c. analysis of extracted serum samples obtained after nifedipine administration showed only a single peak. Any metabolites of nifedipine were not detected in the serum. A biexponential decay of serum nifedipine concentrations with time was observed in non-pregnant rats (Figure 1) and pregnant rats (data not shown). Using the curve-fitting programme, the kinetic parameters given in Table 1 were calculated. Kinetic parameters were found to be similar between non-pregnant and pregnant rats.

Infusion The concentration of nifedipine in the infusate was assayed at the start and end of the infusion in order to monitor possible losses of nifedipine due to photodecomposition which might invalidate the calculation of kinetic parameters. However, no loss of nifedipine over 300 min could be detected.

Serum concentrations of nifedipine produced by infusion were similar at all times between non-pregnant and pregnant rats (Figures 3 and 4). Serum nifedipine concentrations rose during infusion to values of 5.4 ± 0.4 $\mu g\,ml^{-1}$ in non-pregnant rats and 5.3 ± 0.7 $\mu g\,ml^{-1}$ in pregnant rats by 300 min. However, steady-state concentrations were not achieved during the period of infusion. The concentration of nifedipine in foetal serum of

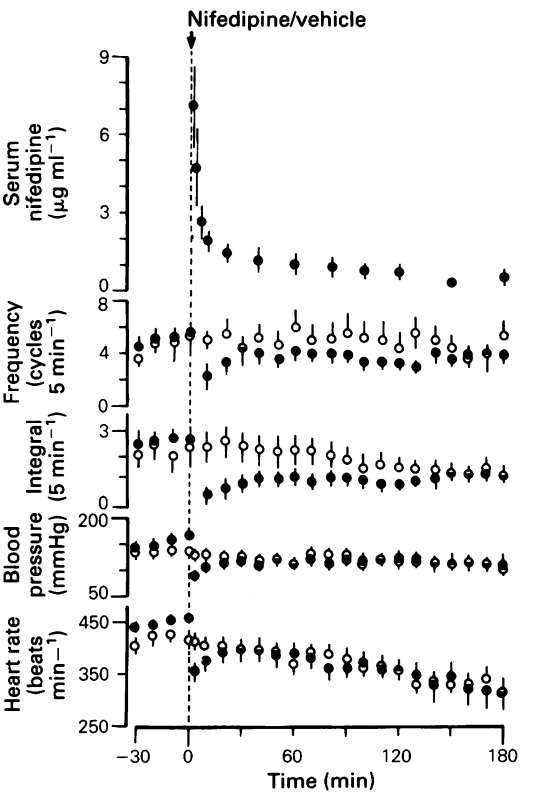

Figure 1 Serum nifedipine concentrations, frequency and integral of uterine contractions, mean blood pressure and heart rate for 30 min before and 180 min after injection of either nifedipine, 400 $\mu g\,kg^{-1}$, (9 rats, ●) or 0.1 ml vehicle (10 rats, ○) to non-pregnant, ovariectomized rats. Values are means with s.e.mean shown by vertical lines.

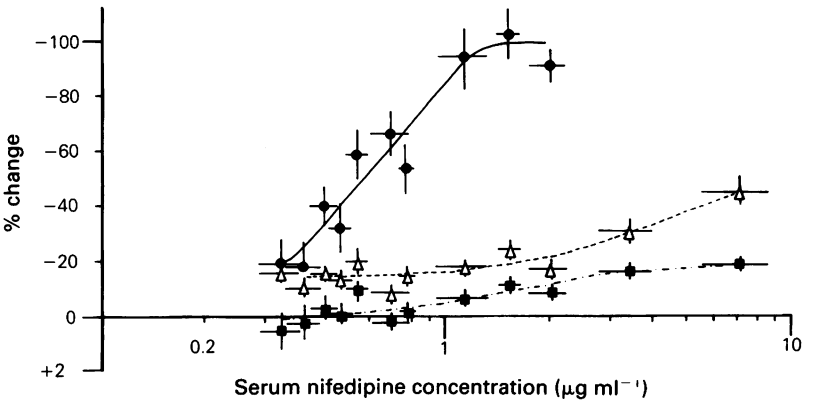

Figure 2 $\log_{10}$ serum nifedipine concentration-effect curves for integral of uterine contractions (●), mean blood pressure (△), heart rate (■) in non-pregnant ovariectomized rats ($n = 9$) derived from bolus dose experiments. Any reduction in these parameters resulting from prolonged anaesthesia and repeated blood sampling observed in control animals has been subtracted from those seen in nifedipine treated animals. Concentrations represent the mean nifedipine concentration ± s.e.mean at each time of blood sampling. Percentage change represents the mean effect ± s.e.mean at the same time point. Standard errors are therefore shown in two directions. Curves were fitted to data by polynomial curve fitting (Sokal & Rohlf, 1969).

$1.5 \pm 0.2\,\mu g\,ml^{-1}$ was lower than in maternal serum at 300 min.

Values for serum nifedipine concentrations during infusion, calculated by use of parameters derived from bolus dose data, were similar to those actually obtained during infusion for the first 180 min (Figures 3 and 4). Thereafter predicted values underestimated experimentally produced serum nifedipine concentrations.

Concentration-effect relationships

Bolus dose; non-pregnant rats The frequency and integral of uterine contractions were markedly reduced at 5 min after nifedipine injection, compared with pre-injection values and in vehicle treated controls (Figure 1). The integral of uterine contractions remained significantly lower than those of controls for up to 35 min after injection ($P < 0.05$). Significant depressions in mean blood pressure and heart rate of 45% and 19% respectively were observed at 1 min after nifedipine injection ($P < 0.05$ compared to vehicle treated controls). A rapid return to control values was observed in both cardiovascular parameters over the next 30 min. A steady decline in all parameters was observed in control animals, probably due to prolonged anaesthesia and repeated blood sampling. The concentration-effect curves

Table 2 Serum nifedipine concentration-effect relationships: IC_{15} values for inhibition of integral of uterine contractions, mean blood pressure and heart rate derived from bolus dose and infusion data

Parameter	*Bolus dose non-pregnant rats*	*Infusion non-pregnant rats*	*Infusion pregnant rats*
Uterus (integral)	0.3 ± 0.05 (9)^{a,c}	0.7 ± 0.1 (10)^{a,b,d}	0.5 ± 0.1 (5)^{b,f,e}
Blood pressure	0.8 ± 0.3 (5)*	-*	0.9 ± 0.3 (5)^{f}
Heart rate	3.8 ± 1.0 (6)*^{,c}	2.8 ± 0.6 (8)*^{,d}	1.2 ± 0.3 (5)^{e}

Values ($\mu g\,ml^{-1}$) are means ± s.e.mean with number of samples per mean given in parentheses.
^{a,b} Significantly different, two-tailed Student's t test: $^{a}P < 0.0005$; $^{b}P < 0.05$.
$^{c-f}$ Means bearing the same superscripts are significantly different, analysis of variance followed by Student's t test; $^{c,d,e}P < 0.01$, $^{f}P < 0.05$. These analyses were also performed on $-\log_{10}$ M concentrations, which are usually normally distributed whereas the untransformed concentrations are not. Significant differences between the same groups were observed after statistical analysis on transformed IC_{15} concentrations.
* No consistent inhibitory effect was detected (blood pressure, bolus dose, 4/9 rats; heart rate, bolus dose 3/9 rats; blood pressure, infusion 10/10 rats; heart rate, infusion 2/10 rats) and concentration-effect curves could not be constructed for these animals.

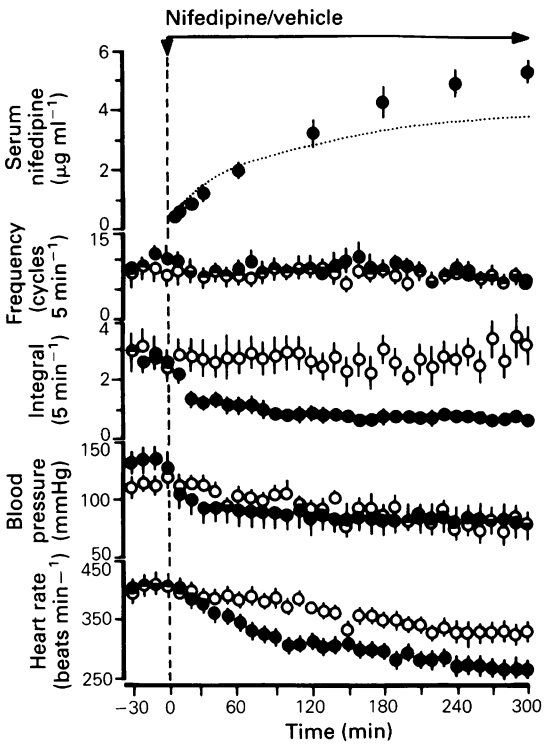

Figure 3 Serum nifedipine concentrations, frequency and integral of uterine contractions, mean blood pressure and heart rate in non-pregnant ovariectomized rats for 30 min before and during 300 min infusion of either nifedipine ($n = 10$, ●) at $10\,\mu g\,kg^{-1}\,min^{-1}$ or vehicle ($n = 10$, ○) at $0.0069\,ml\,min^{-1}$. Values are means with s.e.mean shown by vertical lines. Line (.....) indicates predicted serum concentrations during infusion using kinetic parameters from bolus dose data.

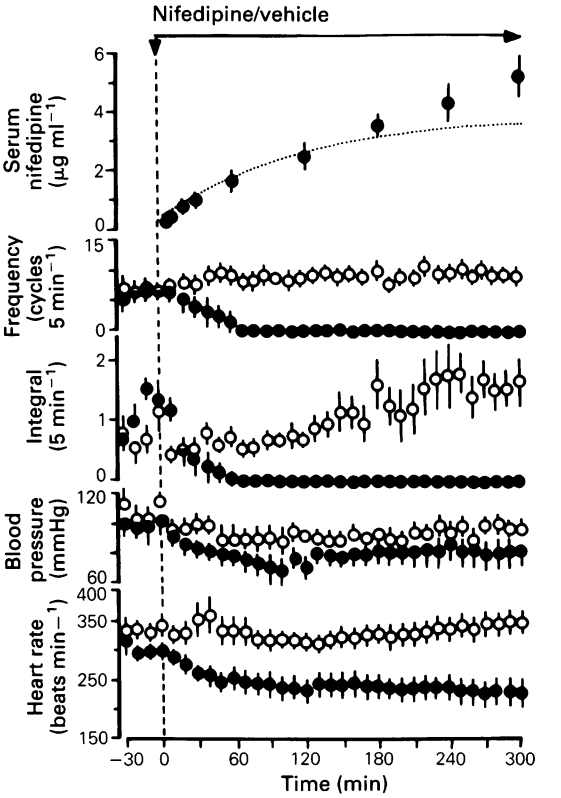

Figure 4 Serum nifedipine concentrations, frequency and integral of uterine contractions, mean blood pressure and heart rate in progesterone-treated ovariectomized pregnant rats for 30 min before and during 300 min infusion of either nifedipine ($n = 5$, ●) at $10\,\mu g\,kg^{-1}\,min^{-1}$ or vehicle ($n = 5$, ○) at $0.0069\,ml\,min^{-1}$. Values are means with s.e.mean shown by vertical lines. Line (.....) indicates predicted serum concentrations during infusion using kinetic parameters from bolus dose data.

derived from these animals are shown in Figure 2. There were positive correlations between inhibition of the three parameters and $\log_{10}$ serum concentrations of nifedipine. The maximum effect on the uterus of 100% inhibition was greater than the maximum effects observed on blood pressure and heart rate. Nifedipine showed significantly greater potency for inhibition of uterine contractions compared to cardiovascular effects ($P < 0.01$) (Table 2.) Inhibition of uterine contractions was observed in all animals. However, in 4/9 and 3/9 rats, no consistent depression of blood pressure or heart rate respectively could be detected beyond 1 min after bolus injection and concentration-effect curves could not be constructed for these animals.

Infusion; non-pregnant rats The frequency of uterine contractions showed little change during nifedipine infusion compared to control pre-infusion values and frequency was similar between nifedipine- and vehicle-infused animals throughout the infusion period (Figure 3). The integral of uterine contractions, however, declined significantly by 15 min after onset of nifedipine infusion and remained significantly lower compared to values in vehicle-infused rats throughout the remainder of the infusion period ($P < 0.001$). The reduction in integral was due to inhibition of amplitude of contractions. No difference in mean blood pressure was observed between nifedipine- and vehicle-treated rats throughout the infusion period. Heart rate, however, was significantly ($P < 0.02$) reduced by nifedipine infusion by 45 min after the start of infusion and remained significantly lower than control vehicle infused values for the remainder of the infusion period ($P < 0.0002$).

Maximum inhibitions of approximately 70% for integral of uterine contractions and 15% for heart rate during nifedipine infusion were observed. There were positive correlations between inhibition of uterine contractions and heart rate and $\log_{10}$ serum concentrations of nifedipine (Figure 5). Nifedipine showed significantly greater potency for inhibition of uterine contractions than for depression of heart rate ($P < 0.01$, Table 1). Nifedipine was less potent against uterine contractions when administered by infusion than after i.v. bolus dose but there was no difference in the potency of nifedipine in the heart with these two methods of administration (Table 2).

Infusion; pregnant rats Both frequency and amplitude of uterine contractions declined rapidly after onset of nifedipine infusion until uterine contractions were completely abolished by 70 min (Figure 4). Uterine quiescence was maintained for the remainder of the infusion period. Mean blood pressure and heart rate also declined significantly during nifedipine infusion and maximum reduction of 30% in both parameters was observed. There were positive correlations between inhibition of uterine contractins, blood pressure and heart rate and $\log_{10}$ serum concentrations of nifedipine (Figure 5). IC_{15} values indicated a greater potency for inhibition of uterine contractions compared to depression of blood pressure ($P < 0.05$) and heart rate ($P < 0.01$) (Table 2). IC_{15} values showed that nifedipine was significantly more potent against uterine contractions in pregnant rats than in non-pregnant rats during i.v. infusion (Table 2).

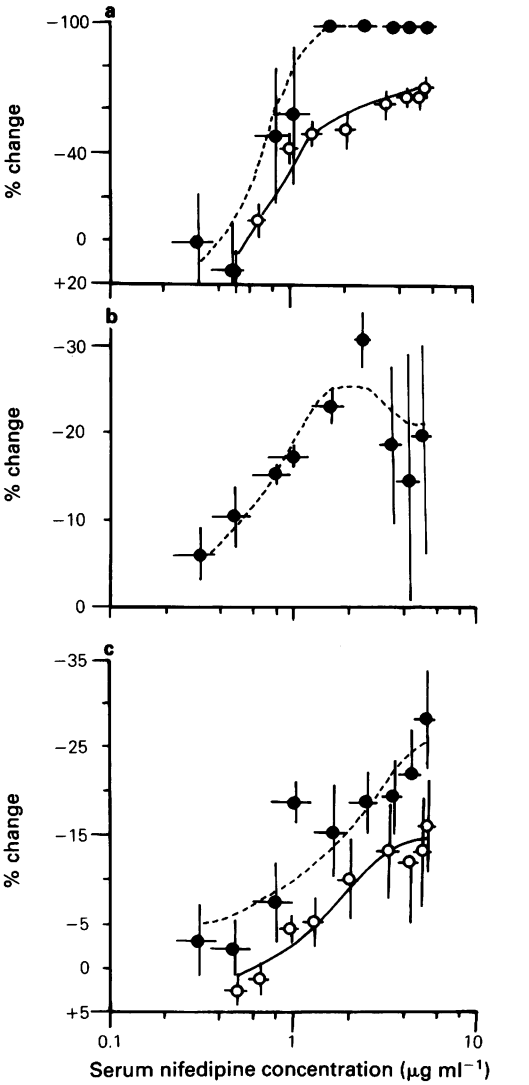

Figure 5 $\log_{10}$ serum nifedipine concentration-effect curves for (a) integral of uterine contractions (10 non-pregnant rats ○, 5 pregnant rats ●); (b) mean blood pressure (5 pregnant rats ●); (c) heart rate (10 non-pregnant rats ○, 5 pregnant rats ●) derived from infusion experiments. Concentrations represent the mean nifedipine concentration ± s.e.mean at each time of blood sampling. Percentage change represents the mean effect ± s.e.mean at the same time point. Standard errors are therefore shown in two directions. Curves were fitted to data by polynomial curve fitting (Sokal & Rohlf, 1969).

Discussion

Pharmacokinetics

The kinetics of nifedipine were similar in non-pregnant and pregnant rats. Nifedipine exhibited a biexponential decay of serum concentrations with time after a bolus i.v. dose in both non-pregnant and late pregnant rats, comparable to the observations in man (Kleinbloesem *et al.*, 1984). Serum concentrations of nifedipine produced by infusion of $10\,\mu g\,kg^{-1}\,min^{-1}$ were also similar at all times in both non-pregnant and pregnant rats.

The major route of elimination of nifedipine is likely to be by hepatic metabolism since only trace amounts of unchanged nifedipine can be detected in urine after oral dose in rat and man (Kleinbloesem *et al.*, 1984; Schlossmann *et al.*, 1975). Although liver weight has been reported to increase during pregnancy (Otway & Robinson, 1968) and progesterone treatment has been shown to increase hepatic demethylation in the rat (Hall *et al.*, 1971), the data suggests that elimination clearance by metabolism of nifedipine is not markedly altered in the pregnant rat. This should be contrasted with diltiazem,

another calcium entry blocker, for which there is increased elimination clearance in pregnant rats (Downing *et al.*, 1987).

Predicted values of serum nifedipine concentrations during infusion using kinetic parameters derived from bolus dose data were close to experimentally derived serum concentrations for 180 min of infusion (Figures 3 and 4). This close fit suggests that the 2-compartment model is a reasonable representation of the handling of nifedipine. After 180 min predicted values underestimated experimental values. Elimination clearances in the infused animals were less than those in the bolus dose experiments for both non-pregnant and pregnant rats. There are a number of possible explanations for this difference. The infused animals received a more prolonged period of anaesthesia. Also, the infused animals received a larger total dose of ethanol (0.012 g bolus dose; 0.23 g infusion) and of polyethylene glycol (0.02 g bolus dose; 0.47 g infusion) from the vehicle. Ethanol is known to reduce nifedipine clearance in the rat (Boje *et al.*, 1984). Finally non-linear kinetics of clearance could apply. The experimental design did not distinguish between these possibilities.

In man, serum protein binding of nifedipine has been reported to be 96–99% (Kleinbloesem *et al.*, 1987; Otto & Lesko, 1986). Nifedipine is as extensively protein bound in rat serum (Raemsch, personal communication). The concentrations of serum albumin, one of the major nifedipine binding proteins (Otto & Lesko, 1986), and total serum protein decline during late pregnancy (Stock *et al.*, 1980). Total plasma volume increases during late pregnancy in the rat (Atherton *et al.*, 1982) ensuring that the total mass of albumin in the circulation changes relatively little. However, for a drug as extensively protein-bound as nifedipine, a relatively small change in serum binding protein concentration would have a marked effect on serum unbound drug concentrations. An increase in unbound fraction of the drug in serum might be accompanied by an increase in elimination clearance resulting in decreased serum total drug concentration (Chou & Levy, 1984; Levy, 1984). No significant differences in kinetic parameters of total serum drug were observed between pregnant and non-pregnant rats. However, changes in kinetics of unbound drug occurring in response to a small change in serum binding of the drug might not be readily detectable. The assay method was not sensitive enough to measure unbound nifedipine concentrations achieved in these animals.

Nifedipine was able to distribute into the foetal compartment although foetal serum concentrations had not reached those of maternal serum by 300 min. It is probable that the rise in foetal serum nifedipine concentrations lags behind the increase in maternal serum nifedipine concentrations because of the time required for placental transfer of the drug. Foetal serum nifedipine concentrations would, therefore, not approach those observed in the maternal serum until after steady-state conditions prevailed in the latter compartment.

Mechanism of action

Nifedipine administration was observed to produce a marked inhibition of uterine contractions after a bolus dose and a sustained inhibition during infusion. The rapid inhibition of uterine contractions after bolus i.v. administration and the positive correlation between serum nifedipine concentrations and the percentage reduction in contractions suggest that the inhibitory effect of nifedipine *in vivo* was due to the parent drug and not a metabolite. In the rat the predominant metabolite of nifedipine is 2-hydroxymethyl-5-methoxycarbonyl-6-methyl-4-(2-nitrophenyl)-pyridine-3-carboxylic acid (Schlossman *et al.*, 1975). Since this metabolite does not contain the intact dihydropyridine structure, it is considered to be relatively impotent.

Nifedipine appears to be less potent *in vivo* against uterine contractions (IC_{15} of 0.3–0.7 $\mu g\,ml^{-1}$) than *in vitro* (IC_{50} of 0.2–5.0 $ng\,ml^{-1}$ dependent on the experimental protocol) (Granger *et al.*, 1985; Downing *et al.*, unpublished). However, serum protein binding of nifedipine would markedly reduce the concentration of unbound drug, which is the form presumed to act on the myometrium. If it is assumed that the unbound drug concentration is about 2% of total serum concentration (Otto & Lesko, 1986; Kleinbloesem *et al.*, 1987; Raemsch, personal communication), then the effective *in vivo* IC_{15} would be in the order of 6–14 $ng\,ml^{-1}$. The latter approaches the potency of nifedipine observed *in vitro*. These data support the idea that the *in vivo* effect is a consequence of the calcium entry blockade described *in vitro*.

Selectivity

For both non-pregnant and pregnant rats, nifedipine was slightly more potent as an inhibitor of uterine contractions than as a vasodepressor or a depressant of heart rate. Furthermore, whereas uterine contractions were inhibited by 70–100%, maximum depressions of 30–45% only were produced in blood pressure and heart rate. These results, therefore, extend the observations of uterine selectivity found in earlier work based on dose-effect data (Abel & Hollingsworth, 1985) to serum concentration-effect data. It is probable that the reflex tachycardia normally observed in conscious animals (Abel & Hollingsworth, 1985) was suppressed by anaesthesia in

the present study and therefore only a small, direct bradycardia was observed.

Potency: pregnant versus non-pregnant rats

Whereas in non-pregnant rats, infusion of nifedipine produced a maximum reduction in uterine contractions of 70%, nifedipine infusion into progesterone-treated pregnant rats, which produced similar serum concentrations to those observed in non-pregnant animals, stopped the uterine contractions. The increased maximum effect and greater potency of nifedipine observed in pregnant rats suggests that physiological changes commensurate with pregnancy may potentiate nifedipine. There are several possible mechanisms by which this potentiation may be achieved: pregnancy-induced changes in serum binding of the drug, pregnancy-induced changes in myometrial receptors for the drug and/or functional synergism between pregnancy-specific hormones and calcium entry blockers in modulating myometrial intracellular calcium concentrations. The possible pregnancy-induced reductions in protein binding of nifedipine have already been referred to above but since unbound drug concentrations were not measured, it is not possible to say whether uterine sensitivity, expressed in terms of unbound drug concentration, was altered in pregnancy.

An increased potency of nifedipine against uterine contractions could also be due to an increase in myometrial dihydropyridine binding sites. An increase in potency of verapamil against uterine contractions *in vitro* has been observed in tissue from rats treated with oestrogen and progesterone, compared to uteri from untreated rats (Ishii *et al.*, 1986), although the number of specific uterine binding sites for [^{3}H]-nitrendipine did not differ between tissues from untreated and hormone-treated animals. Recently Batra (1987) has described an increase in the number of [^{3}H]-nitrendipine binding sites in the uteri of oestrogen-treated rats.

Another possible mechanism for the change in potency of nifedipine is via synergism with ovarian hormones in reducing intracellular calcium concentration in uterine smooth muscle cells. It has been shown that progesterone promotes calcium binding to uterine sarcoplasmic reticulum membranes (Carsten, 1974; 1979), thus reducing intracellular free calcium concentration. When the influx of extracellular Ca^{2+} into the myometrial cell is inhibited by nifedipine, intracellular free calcium concentrations could remain insufficient to initiate contraction. Such a mechanism could explain the increased maximum effect of nifedipine in progesterone-treated pregnant rats compared to non-pregnant, untreated rats. Further work is required to determine the relative importance of changes in serum binding, receptors and hormonal influences on the potency of the calcium entry blockers during pregnancy.

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