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Diltiazem pharmacokinetics in the rat and relationship between its serum concentration and uterine and cardiovascular effects

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1 The kinetics of diltiazem were investigated in ovariectomized (ovx) non-pregnant and intact late pregnant anaesthetized rats following a bolus i.v. injection (2 mg kg^{-1}) and during a 180 min i.v. infusion (50 $\mu g\ kg^{-1}\ min^{-1}$ and 100 $\mu g\ kg^{-1}\ min^{-1}$). Uterine contractions, mean blood pressure and heart rate were measured in the non-pregnant rats.

2 Measurement of serum diltiazem concentrations after bolus i.v. injection in ovx non-pregnant rats showed a biexponential decay with time from which the following parameters were calculated: volume of distribution area ($V_{(area)}$) – 256 ± 46 ml; rate constants k_{12} – 0.46 ± 0.10 min^{-1}; k_{21} – 0.09 ± 0.01 min^{-1}; k_{el} – 0.13 ± 0.03 min^{-1}; elimination clearance – 3.2 ± 0.3 ml min^{-1}; distribution $t_{\frac{1}{2}}$ ($t_{\frac{1}{2}\alpha}$) – 1.4 ± 0.3 min; elimination $t_{\frac{1}{2}}$ ($t_{\frac{1}{2}\beta}$) – 61.2 ± 13.0 min. In pregnant rats, a biexponential decay was also observed with similar parameters to those in non-pregnant animals except for markedly increased $V_{(area)}$ – 1004 ± 184 ml; k_{el} – 0.54 ± 0.16 min^{-1} and elimination clearance – 14.8 ± 2.3 ml min^{-1}.

3 Measurement of serum diltiazem concentrations during infusion yielded the following parameters in non-pregnant ovx rats: $V_{(ss)}$ — 79 ± 10 ml; rate constants k_{12} – 1.02 ± 0.21 min^{-1}; k_{21} – 0.03 ± 0.01 min^{-1}; k_{el} – 0.39 ± 0.06 min^{-1}; elimination clearance – 7.8 ± 1.2 ml min^{-1}. In pregnant rats a marked increase was observed in k_{el} – 1.25 ± 0.38 min^{-1} and elimination clearance – 36.4 ± 13.8 ml min^{-1}.

4 An immediate reduction in uterine contractions, mean blood pressure and heart rate was observed after bolus i.v. injection of diltiazem with a return towards control values as serum diltiazem concentrations declined. There were significant correlations between the inhibition of the 3 parameters and the log serum concentrations of diltiazem. Serum concentration-response curves indicated IC_{50} values of 0.5 $\mu g\ ml^{-1}$ for inhibition of uterine contractions, 0.7 $\mu g\ ml^{-1}$ for reduction in blood pressure and 1.2 $\mu g\ ml^{-1}$ for reduction in heart rate. There were maintained reductions in the integral of uterine contractions, mean blood pressure and heart rate during infusion.

5 The metabolite desacetyldiltiazem was rarely detected after i.v. bolus injection and was not found in 5/13 rats infused with diltiazem, yet significant inhibition of uterine contractions was observed in all rats. Diltiazem was 3.2 fold more potent than desacetyldiltiazem as an inhibitor of contractions of the rat isolated uterus.

6 These findings indicate that the inhibition of uterine contractions is due to a direct action of diltiazem, and not the metabolite desacetyldiltiazem, and suggest only a slight selectivity for uterine inhibition compared to cardiovascular effects.

Introduction

Development of tension in uterine smooth muscle is dependent in part upon the passage of extracellular calcium ions into the myometrial cell via voltage-dependent channels in the plasma membrane (Edman & Schild, 1962; Bolton, 1979). A group of structurally diverse compounds known as calcium (Ca^{2+}) entry blockers will inhibit Ca^{2+} movement via these channels. A number of these compounds have been shown to be potent inhibitors of tension development by uterine smooth muscle *in vitro* (Granger *et al.*, 1985; 1986; Edwards *et al.*, 1986). Gallopamil, verapamil, (+)-*cis*-diltiazem (hereafter referred to as diltiazem) and

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nifedipine also inhibited uterine contractions *in vivo* in the ovariectomized (ovx) *post-partum* rat after bolus i.v. injection (Abel & Hollingsworth, 1985) and the latter two compounds produced sustained inhibition of uterine contractions during i.v. infusion (Abel & Hollingsworth, 1986a). Diltiazem and nifedipine were able to prevent preterm delivery when administered by infusion to ovx, oestrogen-treated pregnant rats (Abel & Hollingsworth, 1986b).

There is, however, limited evidence that the observed relaxant effects of the Ca^{2+} entry blockers on uterine smooth muscle *in vivo* are due to a direct action of the parent drugs on the myometrium. It is not known if the serum concentrations required for inhibition of uterine contractions *in vivo* are comparable to those required for the same effect *in vitro*. Additionally, the physiological changes commensurate with advancing pregnancy, such as increased blood volume, peripheral compartment volume, renal clearance, etc, markedly affect drug pharmacokinetics. It is, therefore, important to determine how the kinetics of the drug differ in late pregnancy compared to the non-pregnant condition.

The pharmacokinetics of one Ca^{2+} entry blocker, diltiazem, has been studied in detail in ovx, non-pregnant and intact late pregnant rats following bolus i.v. injection and infusion. To demonstrate the direct action of diltiazem on myometrial smooth muscle, we have examined the relationship between serum concentrations of diltiazem and a pharmacologically active metabolite, desacetyldiltiazem (Yabana *et al.*, 1985), and their effects on the uterus and cardiovascular system in the non-pregnant rat. Preliminary results have been demonstrated to a joint meeting of the Belgian, British and Dutch Pharmacological Societies (Downing *et al.*, 1986).

Methods

Thirty-six non-pregnant (200–250 g) and 16 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.

Non-pregnant rats

The rats were anaesthetized with tribromoethanol (240 mg kg^{-1}, i.p.), subjected to bilateral ovariectomy via ventral laparotomy and a small latex, pressure-recording balloon was inserted into one uterine horn. The balloon catheter was passed subcutaneously to the back of the neck where it was exteriorized and protected by a long metal spring which allowed the animal almost unrestricted movement within the cage. Distilled water (0.1 ml) was placed in the balloon via the balloon catheter and the catheter connected to a fluid filled EM750 Elcomatic or P23Db Statham pressure transducer. Intra-uterine pressure was recorded on a 4-channel polygraph (Grass Instruments, Quincy, MA, U.S.A.). A uterine contraction cycle was defined as a rise and fall of >5 mmHg. Uterine contractions were quantified by integrating the area under the pressure curve above basal resting pressure using a 7PIOB integrator. The animals were allowed 24–48 h recovery before being used for either bolus dose or infusion experiments.

Pregnant rats

These rats were used for either bolus dose or infusion experiments without prior ovariectomy or balloon implantation.

Bolus dose

The rats were anaesthetized with tribromoethanol, the right carotid artery (non-pregnant only) and right jugular vein cannulated (PP50 polyethylene tubing, Portex Ltd, Hythe, Kent) and a tracheotomy tube inserted (PP100 polyethylene tubing) to ensure maintenance of a clear airway. Anaesthesia was maintained throughout with tribromoethanol. The carotid artery cannula was connected to a fluid filled pressure transducer for measurement of blood pressure and heart rate (Model 7P4F EKG Tachograph preamplifier, Grass Instruments). Uterine contractions, blood pressure and heart rate (non-pregnant rats only) were recorded for 30 min before and 120 min after i.v. injection of either diltiazem (2 mg kg^{-1} in 0.1 ml saline, 9 non-pregnant rats, 8 pregnant rats) or 0.1 ml saline (5 non-pregnant rats). Blood samples, normally 0.2 ml, were taken via the jugular cannula at the following times after bolus i.v. injection: 1, 2, 5, 10, 20, 40, 60, 80, 100, 120 (1 ml sample) min. The blood removed was replaced with the same volume of saline containing 20 iu heparin (Sigma Chemical Company, Poole, Dorset) per ml. The animals were then killed without recovery from anaesthesia.

Infusion

The rats were anaesthetized and blood vessels cannulated as described for bolus dose. In addition, the left jugular vein was cannulated to permit continuous infusion. Uterine contractions, blood pressure and heart rate (non-pregnant rats only) were recorded for 30 min before, during and for 120 min following cessation of infusion of either diltiazem at two rates (50 $\mu g\ kg^{-1}\ min^{-1}$, 5 non-pregnant rats or 100 $\mu g\ kg^{-1}\ min^{-1}$, 8 non-pregnant rats, 8 pregnant rats), or saline (9 non-pregnant rats) at 0.0069 ml min^{-1} (Harvard Compact Infusion Pump, Harvard Apparatus Co, South Natick, MA, U.S.A.) for 180 min. Blood sam-

ples, 0.2 ml, were taken from the right jugular cannula at the following times: 5, 10, 30, 60, 90, 120, 150, 180 min during infusion and 2, 5, 10, 20, 40, 60, 80, 100, 120 min after infusion. The blood removed was replaced with saline containing heparin. The animals were then killed without recovery from anaesthesia.

All blood samples were placed immediately on ice until centrifuged at 15,600 *g* (MSE microcentaur centrifuge) for 10 min and the serum collected. The serum samples were stored at −20°C until assayed for diltiazem by high performance liquid chromatography (h.p.l.c.). All samples were assayed within 7 days.

High performance liquid chromatography

The method for assay of diltiazem was based on the extraction method of Wiens *et al.* (1984) and the h.p.l.c. method of Verghese *et al.* (1983). Briefly, 100–1000 μl samples of serum, to which 750 ng verapamil internal standard and 100 μl 0.1 M sodium borate buffer, pH 9.0, had been added were extracted with 3.5 ml or 7 ml hexane:isopropanol, 98:2, by vortex mixing for 4 min. Diltiazem and desacetyldiltiazem standards (10, 25, 50, 100, 250, 500, 800, 1000 ng) and 750 ng verapamil internal standard were added to blank rat plasma and extracted as described for the experimental samples. The aqueous layer was frozen with dry ice/methanol and the organic layer decanted into 7 ml conical tipped tubes. The hexane/isopropanol was dried down at 55°C under oxygen-free nitrogen. The tubes were rinsed with 2 × 100 μl hexane/isopropanol, again dried down under O_2-free nitrogen, and the extract taken up in 80 μl mobile phase (0.05 M ammonium dihydrogen phosphate:methanol:triethylamine, 55:45:0.5, pH 5.0). Fifty μl of the extract were assayed and the ratio of heights of diltiazem and metabolite peaks:peak height of internal standard at 237 nm wavelength was determined. Assays were performed within 48 h of extraction as significant loss of diltiazem (approximately 50%) was experienced due to adhesion of the drug to glassware when samples were stored for longer than 48 h. Loss of sample from serum during storage at −20°C was not significant for up to 3 months. Blank serum was also extracted and chromatographed before the standards and samples. This provided a baseline which was then subtracted from all succeeding chromatographs.

The h.p.l.c. system consisted of a Varian 5500 solvent pump, automatic sampler, UV200 monitor, and a Varian Vista 402 integrator and printer (Varian, Walnut Creek, CA, U.S.A.). The columns used were a 25 cm × 4.6 mm Zorbax CN (6.5 μm) reversed-phase column with a CN pellicular guard column (Technicol, Stockport, Cheshire). H.p.l.c. grade solvents (Rathburn Ltd, Walkerburn, Peebleshire) and Analar grade reagents were used throughout.

Sensitivities of the assay were taken as the smallest amounts of standard diltiazem and desacetyldiltiazem which could be measured with a coefficient of variation (CV) of less than 20%. Recovery, intra- and inter-assay variation and accuracy were determined at 3 doses of diltiazem and desacetyldiltiazem. Standard diltiazem and desacetyldiltiazem (50, 250, 1000 ng) were added to blank serum, extracted and assayed as described. The same doses of diltiazem and desacetyldiltiazem were assayed in mobile phase without prior extraction from blank serum. Recoveries were taken as the peak heights of extracted diltiazem and desacetyldiltiazem as a percentage of peak heights of non-extracted standards. Intra- and inter-assay variation were expressed as the coefficient of variation of the mean extracted standards at each dose within one assay and between assays performed over a period of 3 months, respectively. Accuracy is presented as the mean value of extracted standards estimated by the assay at each dose.

Pharmacokinetic analysis

Initial analysis of data after bolus dose assumed a biexponential decay of serum concentrations of diltiazem against time (see Results). 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. From these intercepts and slopes, the following kinetic parameters were calculated: distribution $t_{\frac{1}{2}}$ ($t_{\frac{1}{2}\alpha}$), elimination $t_{\frac{1}{2}}$ ($t_{\frac{1}{2}\beta}$), rate constants (k_{12}, k_{21}, k_{el}), area under curve (AUC) = (A/α) + (B/β), elimination clearance (CL_{el}) = dose/AUC, volume of distribution area (V_{area}) = elimination clearance/β. AUC was also calculated by the trapezoidal method but values did not differ significantly.

For the infusion experiments a two-compartment open system was assumed. The rise during infusion and decline post infusion of serum diltiazem concentrations were fitted by a Nelder and Mead non-linear optimization programme (Box *et al.*, 1969). This calculated the volumes of and clearance between the two compartments and the elimination clearance. Rate constants were calculated from clearances divided by the volumes of the appropriate compartment (Notari, 1980).

Concentration-effect relationship

The % change in integral of uterine contractions compared to pretreatment values was determined at 5 min after diltiazem injection and at every subsequent time of blood sampling for each animal. The % changes in blood pressure and heart rate compared to pretreat ment values were determined at 1 min after bolus

injection and every subsequent time of blood sampling. Thus, for each animal, the serum concentration of diltiazem and the change in biological parameters were determined at the same time points.

Statistics

Means of integral of uterine contractions, blood pressure and heart rate were compared between treatment groups using analysis of variance followed by 2-tailed Student's *t* test (Sokal & Rohlf, 1969). Curves were fitted to concentration-effect data using polynomial curve-fitting equations (Sokal & Rohlf, 1969).

Sources of drugs

(+)-*cis*-Diltiazem hydrochloride, desacetyldiltiazem and N,*O*-demethyldesacetyldiltiazem were supplied by Synthelabo (Paris, France). Verapamil hydrochloride was supplied by Knoll (Ludwigshafen, W. Germany).

Results

High performance liquid chromatography

Details of recovery, accuracy and sensitivity of the assay method are given in Table 1. The assay showed a high recovery of diltiazem, desacetyldiltiazem and verapamil from serum, was precise (intra- and inter-assay CV $< 10\%$) and showed no bias (accuracy – mean deviation from expected value $< 8\%$). Figure 1 shows typical chromatographs of diltiazem, desacetyldiltiazem and verapamil standards and extracted serum from a rat given an infusion of diltiazem. Diltiazem (retention time 6.8 min) was clearly separated from desacetyldiltiazem (retention time 5.2 min). Peaks at 6.8 min and 5.2 min detected in extracted serum from a rat infused with diltiazem were taken to be diltiazem and desacetyldiltiazem, respectively, as they were enhanced by the addition of standard diltiazem and desacetyldiltiazem. The serum concentrations of diltiazem obtained from bolus i.v. injection or during infusion were always in excess of the sensitivity of the assay (50 ng per tube, 50 ng ml^{-1}). Figure 2 shows the calibration of peak height ratios of increasing amounts of diltiazem and desacetyldiltiazem relative to a fixed amount of internal standard. These relationships were linear throughout the range of quantities measured in the study.

Bolus dose

Pharmacokinetics – non-pregnant rats H.p.l.c. analysis of extracted serum samples obtained after bolus i.v. injection of diltiazem (2 mg kg^{-1}) showed only a single peak with a retention time of 6.8 min for the majority of rats. Desacetyldiltiazem was rarely detected in samples after bolus dose. A biexponential decay of serum diltiazem concentrations with time was observed (Figure 3). Using a curve fitting programme, the kinetic parameters given in Table 2 were calculated. The initial distribution phase was rapid ($t_{1/2\alpha}$ of 1.4 ± 0.3 min) and therefore difficult to measure accurately. The elimination phase had a half-life of 61.2 ± 13.0 min. A large volume of distribution (V_{area}) of 256 ± 46 ml was observed for diltiazem.

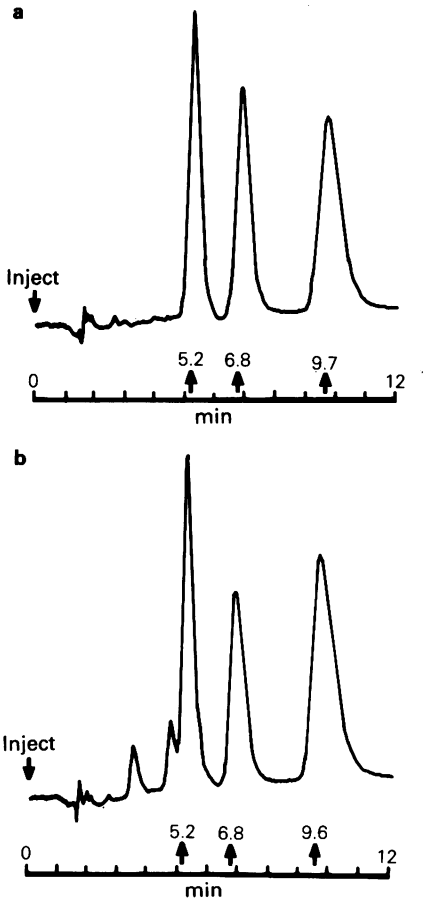

Figure 1 High performance liquid chromatography traces of (a) extracted blank rat serum containing 250 ng diltiazem standard (6.8 min), 250 ng desacetyldiltiazem standard (5.2 min) and 750 ng verapamil internal standard (9.7 min), (b) extracted serum from a non-pregnant rat given an infusion of diltiazem at 100 $\mu g\ kg^{-1}\ min^{-1}$ for 180 min. Peaks appearing at 5.2 min and 6.8 min are taken to be desacetyldiltiazem and diltiazem, respectively.

Table 1 Details of the high performance liquid chromatography assay of diltiazem and desacetyldiltiazem

Parameter	*Diltiazem*	*Desacetyldiltiazem*	*Verapamil*
Retention time	6.7 (1.7%) min	5.2 (1.5%) min	9.6 (2.0%) min
Recovery (100 µl)	93.2 (4.2)%	100.6 (3.6)%	95.4 (2.0)%
Recovery (1 ml)	67.2 (8.9)%	109.1 (8.4)%	88.1 (9.7)%
Intra-assay variation			
50 ng	6.5%	9.0%	
250 ng	3.3%	2.5%	
1000 ng	5.2%	4.4%	
Inter-assay variation over 3 months			
50 ng	8.5%	8.6%	
250 ng	2.3%	3.1%	
1000 ng	3.3%	4.5%	
Accuracy			
50 ng	57.2 (5.2%) ng	61.9 (5.3%) ng	
250 ng	247 (1.5%) ng	257 (1.8%) ng	
1000 ng	1001 (2.1%) ng	1010 (2.0%) ng	
Sensitivity	50 ng ml^{-1} (50 ng/tube)	25 ng ml^{-1} (25 ng/tube)	

Values are means (minimum of 8 values) with coefficients of variation in parentheses. Intra-assay variation are means of 3 values.

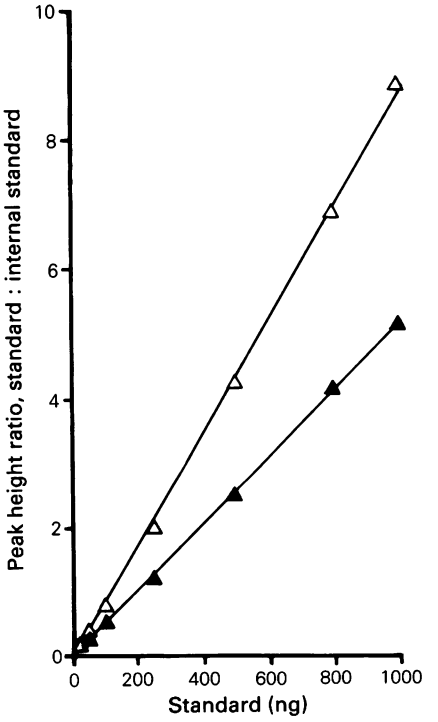

Figure 2 Ratio of peak height of diltiazem (▲) and desacetyldiltiazem (△) standards, 10–1000 ng, extracted from blank rat serum to internal standard peak height.

Pharmacokinetics – late pregnant rats Only diltiazem was detected in the majority of serum samples after bolus i.v. injection of diltiazem (2 mg kg^{-1}). A bi-exponential decay of serum diltiazem concentrations with time was again observed (Figure 4) and the kinetic parameters calculated from this data are given in Table 2. A marked increase (approximately four fold) in several kinetic parameters was observed in late pregnant rats compared to non-pregnant animals. CL_{el} was increased by 4.6 fold, k_{el} by 4.1 fold and V_{area} by 3.9 fold. There were no significant changes in $t_{1\alpha}$, $t_{1\beta}$, k_{12} or k_{21}.

Table 2 Diltiazem — kinetic parameters derived from a bolus dose of 2 mg kg^{-1}

Parameter	*Non-pregnant*	*Pregnant*
V_{area} (ml)	256 ± 46	1004 ± 184†
k_{12} (min^{-1})	0.46 ± 0.10	1.26 ± 0.26
k_{21} (min^{-1})	0.09 ± 0.01	0.09 ± 0.02
k_{el} (min^{-1})	0.13 ± 0.03	0.54 ± 0.16*
CL_{el} (ml min^{-1})	3.2 ± 0.3	14.8 ± 2.3**
$t_{1\alpha}$ (min)	1.4 ± 0.3	0.6 ± 0.2
$t_{1\beta}$ (min)	61.2 ± 13.0	49.0 ± 8.5

Values are means ± s.e.mean (non-pregnant, $n = 8$; pregnant, $n = 7$). One non-pregnant rat was omitted from the analysis. Mean of pregnant rats significantly greater than non-pregnant (*$P < 0.05$; † $P < 0.002$; **$P < 0.001$). V_{area} = volume of distribution area; k_{12}, k_{21} and k_{el} = rate constants; CL_{el} = elimination clearance; $t_{1\alpha}$ and $t_{1\beta}$ = distribution and elimination half-life, respectively.

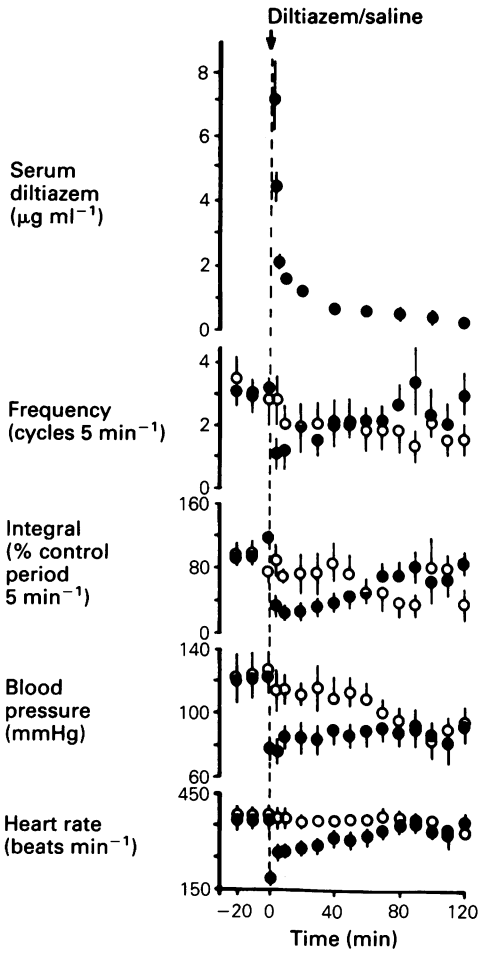

Figure 3 Serum diltiazaem concentrations, frequency and integral of uterine contractions, mean blood pressure and heart rate for 30 min before and 120 min after injection of either diltiazem, 2 mg kg^{-1} to non-pregnant ovariectomized rats (9 rats, ●) or 0.1 ml saline (5 rats, ○). Values are means and vertical lines show s.e.mean. Control saline-treated animals showed low absolute values for integral of uterine contractions, therefore, values for both groups of animals have been normalized by expressing them as % of mean integral for 30 min pretreatment period.

Biological effects – non-pregnant rats Uterine contractions were markedly reduced 5 min after injection of diltiazem ($P < 0.001$ compared to saline controls) (Figure 3). Uterine contractions of diltiazem-treated rats remained significantly lower than those of controls for up to 60 min after injection ($P < 0.02$). Maximum falls in mean blood pressure (30%) and heart rate (25%) were observed at 1 min after diltiazem injection ($P < 0.001$ compared to saline controls). A gradual return to control values occurred over the following 60 min. Blood pressure tended to decline with time in controls, probably as a result of prolonged anaesthesia and repeated blood sampling. Mean reduction in integral uterine contractions, blood pressure and heart rate in control animals with time were subtracted from the reductions in biological parameters in each individual diltiazem-treated rat. Relationships between these corrected changes in integral of uterine contractions, mean blood pressure and heart rate against $\log_{10}$ serum diltiazem concentrations are shown in Figure 5. There were positive correlations between the biological changes and the $\log_{10}$ serum diltiazem concentrations. The serum concentrations required to produce 50% inhibition (IC_{50}) were approximately 0.5 μg ml^{-1} for inhibition of uterine contractions, 0.7 μg ml^{-1} for reduction in mean blood pressure and 1.2 μg ml^{-1} for reduction in heart rate. The data did not permit more precise determination of IC_{50}s.

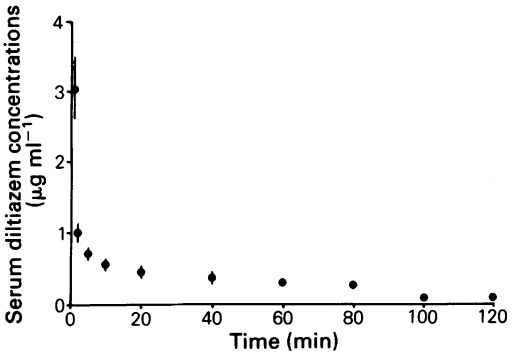

Figure 4 Serum diltiazem concentrations in 8 late pregnant rats after bolus i.v. injection of diltiazem (2 mg kg^{-1}). Values are means with vertical lines indicating s.e.mean.

Infusion

Pharmacokinetics – non-pregnant rats H.p.l.c. analysis of extracted serum samples from 6/8 rats infused with diltiazem (100 μg kg^{-1} min^{-1} for 180 min) showed a profile as in Figure 1b. In addition to the peak at 6.8 min (diltiazem), there was a major peak with a retention time of 5.2 min which co-chromatographed with standard desacetyldiltiazem. There were also minor peaks with retention times of 3.4 and 4.7 min. In 2/8 rats, no peak equivalent to desacetyldiltiazem was observed. However, a peak

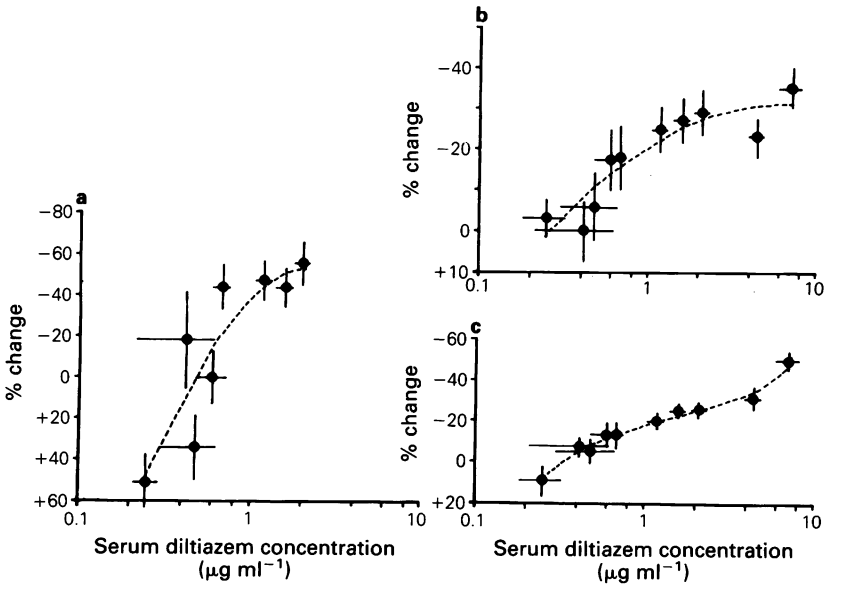

Figure 5 Log_{10} serum diltiazem concentration-effect curves for (a) integral of uterine contractions, (b) mean blood pressure, (c) heart rate. Any reduction in the three parameters observed in control animals resulting from prolonged anaesthesia and repeated blood sampling has been subtracted from those observed in diltiazem-treated animals. Concentrations represent the mean diltiazem concentration with s.e.mean shown by horizontal lines at each time of blood sampling after bolus injection. % change represents the mean effect at the same time point and vertical lines show s.e.mean. Standard errors are therefore shown in two directions.

with a retention time of 5.6 min was observed. It was assumed that this was a metabolite of diltiazem, although little attempt was made to identify it. Authentic N,*O*-demethyldesacetyldiltiazem (a metabolite of diltiazem, Meshi *et al.*, 1971; Yabana *et al.*, 1985) had a retention time of 3.1 min when chromatographed in mobile phase and was not extracted from spiked blank serum by hexane/isopropanol. In animals infused with diltiazem at 50 $\mu g\,kg^{-1}\,min^{-1}$ for 180 min, h.p.l.c. analysis of extracted serum samples from 2/5 rats exhibited major peaks with retention times equivalent to diltiazem and desacetyldiltiazem and from 3/5 rats exhibited major peaks equivalent to diltiazem and the unidentified peak at 5.6 min.

Serum concentrations of diltiazem rose during infusion of diltiazem at 100 $\mu g\,kg^{-1}\,min^{-1}$ to steady-state concentrations (C_{ss}) of $2.2 \pm 0.3\,\mu g\,ml^{-1}$ by 120 min (Figure 6). After cessation of infusion, concentrations declined in a monoexponential manner with time ($t_{\frac{1}{2}}$ of 69.1 ± 10.7 min). Concentrations of desacetyldiltiazem in 6/8 rats rose steadily during the infusion period to $2.2 \pm 0.5\,\mu g\,ml^{-1}$ by 180 min and did not decline significantly after cessation of infusion. Serum concentrations of diltiazem reached a C_{ss} of $0.9 \pm 0.2\,\mu g\,ml^{-1}$ by 120 min during infusion of diltiazem at 50 $\mu g\,kg^{-1}\,min^{-1}$ (Figure 7). However, serum desacetyldiltiazem concentrations attained in 2/5 rats during infusion were not different from those observed during diltiazem infusion at 100 $\mu g\,kg^{-1}\,min^{-1}$. Kinetic parameters derived from infusion data are given in Table 3.

Pharmacokinetics – pregnant rats Serum diltiazem and desacetyldiltiazem concentrations achieved during infusion of diltiazem at 100 $\mu g\,kg^{-1}\,min^{-1}$ are shown in Figure 8. Serum diltiazem concentrations at a steady state of $1.1 \pm 0.2\,\mu g\,ml^{-1}$ were observed. Significant concentrations of desacetyldiltiazem were observed in 6/8 animals, reaching $0.9 \pm 0.2\,\mu g\,ml^{-1}$ by 180 min. Kinetic parameters derived from these data are given in Table 3. In accordance with observations after bolus dose, a marked increase in CL_{cl} and k_{12} was apparent in late pregnant rats compared to non-pregnant rats. A significant increase in clearance from the central compartment was also observed in pregnant rats. A sample of foetal serum was obtained from 7/8 rats at cessation of infusion. Mean foetal serum concentrations of diltiazem of $1.1 \pm 0.2\,\mu g\,ml^{-1}$ were similar to those of maternal serum. However, foetal desacetyldiltiazem concentrations (4/7 rats) of $0.4 \pm 0.2\,\mu g\,ml^{-1}$ were only 40% of maternal serum desacetyldiltiazem concentrations at 180 min.

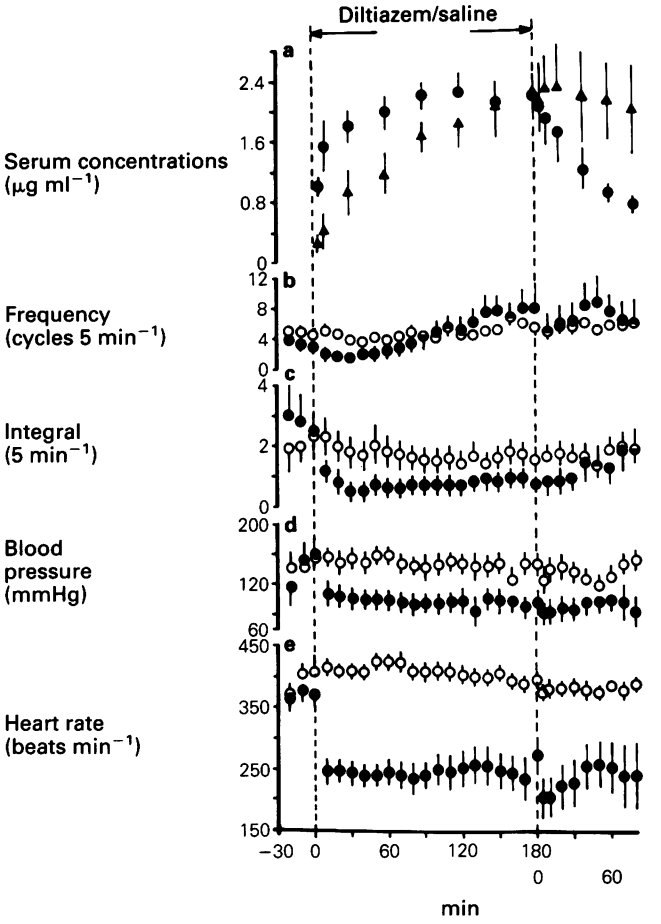

Figure 6 (a) Serum diltiazem (●) and desacetyldiltiazem (▲) concentrations, (b) frequency and (c) integral of uterine contractions, (d) mean blood pressure and (e) heart rate for 30 min before, 180 min during and 120 min after infusion of either diltiazem (8 non-pregnant ovariectomized (ovx) rats, (●) at 100 μg kg^{-1} min^{-1}) or saline (9 non-pregnant ovx rats, (○) at 0.0069 ml min^{-1}). Values are means and vertical lines show s.e.mean. In (a) desacetyldiltiazem concentrations are means of 6 rats.

Biological effects – non-pregnant rats The biological effects observed during diltiazem infusion were similar at both rates of infusion. There was a marked decline in amplitude of contractions during diltiazem infusion but in some rats frequency of uterine contractions was observed to increase concomitantly. However, the net effect was that integral of uterine contractions declined markedly and remained significantly lower than that of control animals during the 180 min diltiazem infusion at both infusion rates (50 and 100 μg kg^{-1} min^{-1}; $P < 0.001$) (Figure 6). A return of uterine contractions to control levels was observed during the post-infusion period. Mean blood pressure and heart rate were reduced by 30% and 35% respectively by 5 min during infusion and this reduction was sustained throughout the infusion period. Mean blood pressure and heart rate did not return to pretreatment values after cessation of infusion, although this may have been due in part to the effects of prolonged anaesthesia and repeated blood sampling. There were no differences in biological effects between those animals in which desacetyldiltiazem was detected and in those rats in which metabolites other than desacetyldiltiazem were observed.

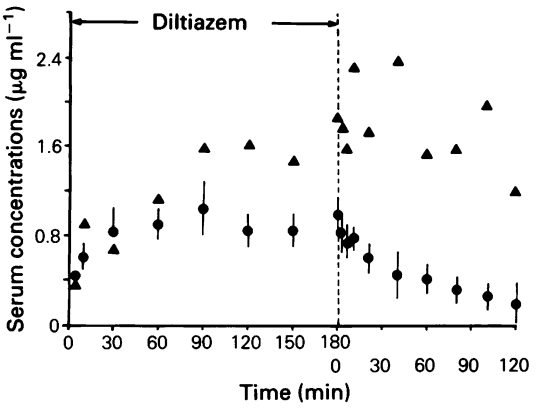

Figure 7 Serum diltiazem (●) and desacetyldiltiazem (▲) concentrations in 5 non-pregnant ovariectomized rats, during infusion of diltiazem at 50 μg kg^{-1} min^{-1} and for 120 min after infusion of diltiazem. Concentrations for diltiazem are means with vertical lines showing s.e.mean. Desacetyldiltiazem concentrations are means of 2 rats.

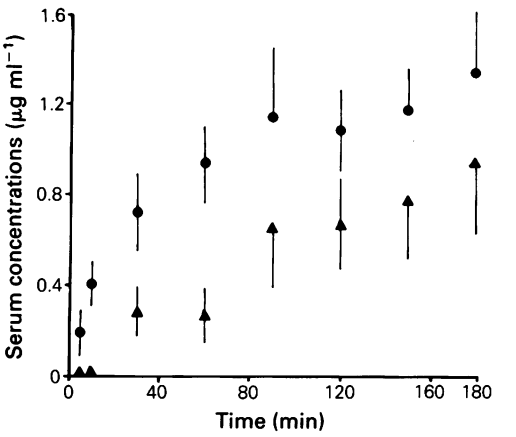

Figure 8 Serum diltiazem (●) and desacetyldiltiazem (▲) concentrations in 8 late pregnant rats during infusion with diltiazem at 100 μg kg^{-1} min^{-1}. Values are means with vertical lines showing s.e.means. Desacetyldiltiazem concentrations are the means of 6 rats.

Isolated uterus

Desacetyldiltiazem was found in the serum of rats during infusion and may contribute to the biological effects of diltiazem. The relative potencies of desacetyldiltiazem and diltiazem as inhibitors of spasms of the isolated uterus of the non-pregnant, ovx rat to K^+ (40 mM) were therefore determined by the technique of Granger *et al.* (1986). The IC_{50} values for diltiazem and desacetyldiltiazem were 6.10 ± 0.07 and 5.60 ± 0.08 ($-\log_{10}$ M, mean ± s.e.mean, $n = 4$) respectively. Diltiazem, therefore, has a potency 3.2 fold that of its metabolite.

Discussion

This study extends previous observations of the effects of diltiazem on the uterus (Abel & Hollingsworth, 1985; 1986a,b). We have now provided evidence that the effects observed on uterine contractions are due to diltiazem and not to its major metabolite desacetyldiltiazem. We have also shown that diltiazem exhibits pharmacokinetics in the rat which can be described by a 2-compartment open model with a rapid distribution phase.

Diltiazem exhibited a biexponential decay of serum concentrations with time after bolus i.v. dose in both

Table 3 Diltiazem — kinetic parameters derived from infusion

Parameter	*Non-pregnant*	*Pregnant*
$V_{central\ compartment}$ (ml)	26 ± 6	43 ± 19
$V_{peripheral\ compartment}$ (ml)	53 ± 9	58 ± 21
Total V_{ss} (ml)	79 ± 10	101 ± 25
Elimination CL (ml min^{-1})	7.8 ± 1.2	36.4 ± 13.8†
k_{12} (min^{-1})	1.02 ± 0.21	1.95 ± 0.38*
k_{21} (min^{-1})	0.03 ± 0.01	0.08 ± 0.04
k_{el} (min^{-1})	0.39 ± 0.06	1.25 ± 0.38†

Values are means ± s.e.mean of 13 non-pregnant rats and 8 pregnant rats. For non-pregnant animals, results obtained by infusion at 50 μg kg^{-1} min^{-1} and 100 μg kg^{-1} min^{-1} have been pooled. Mean of pregnant rats significantly greater than non-pregnant (* $P < 0.05$; † $P < 0.02$). For key to abbreviations used see Table 2. V_{ss} = volume at steady-state.

non-pregnant and late pregnant rats. The elimination clearance of diltiazem is probably due largely to metabolism since negligible concentrations of diltiazem were found in urine and faeces (Meshi *et al.*, 1971). At least 7 metabolites (plus their conjugates) have been detected in urine and bile after oral administration in the rat (Meshi *et al.*, 1971). It was concluded that desacetyldiltiazem was a minor metabolite only and that N,*O*-demethyldesacetyldiltiazem was the main urinary metabolite. In contrast, after i.v. administration, we have detected significant serum concentrations of desacetyldiltiazem in most animals. N,*O*-demethyldesacetyldiltiazem was not extracted by the hexane/isopropanol solvent and we cannot, therefore, comment as to its formation after i.v. administration. Burke (personal communication) has suggested that N-demethyldesacetyldiltiazem (metabolite 2 of Yabana *et al.*, 1985) is the most significant metabolite found in serum in rats after oral administration of diltiazem. It is likely that one of the peaks observed after i.v. administration (3.4, 4.7 or 5.6 min retention time) is due to this substance. The similarity of serum desacetyldiltiazem concentrations in rats given diltiazem infusion at 50 and 100 $\mu g\,kg^{-1}\,min^{-1}$ suggests that the formation of desacetyldiltiazem from the parent drug may be a saturable process in the rat but the numbers of animals involved were very small.

Changes in the pharmacokinetics of diltiazem were observed in late pregnant rats compared to non-pregnant animals and in particular it was found that elimination clearance of diltiazem was increased by approximately four fold. As volume of distribution (V_{area}) was similarly increased so elimination $t_{\frac{1}{2}}$ was unaltered. Infusion of diltiazem at identical dose rates produced serum diltiazem concentrations some 50% lower in pregnant rats than in non-pregnant animals. This may result in reduced effectiveness of a given dose of drug during pregnancy. Hepatic metabolism of drugs has generally been found to show only minor changes during pregnancy (Hytten, 1984; Nau *et al.*, 1984). However, the main system investigated has been the cytochrome P-450-dependent mono-oxygenase system, whereas diltiazem elimination is mainly by deacetylation and demethylation (Meshi *et al.*, 1971). Progesterone treatment has been shown to increase hepatic demethylation in the rat (Hall *et al.*, 1971) and liver weight has been reported to increase by 50% during pregnancy (Otway & Robinson, 1968). These two factors might contribute to an increase in hepatic metabolism of diltiazem. Several fluid compartments (plasma and blood volume, total body water) increase in the rat during pregnancy (Atherton *et al.*, 1982). These changes may in part explain the increase in volume of distribution of diltiazem observed after bolus injection. It is to be noted that values of V_{ss}, calculated from the infusion experiments, were smaller than V_{area}, as anticipated (Notari, 1980). Also, since there was no change in V_{ss} between non-pregnant and pregnant rats, changes in volume of distribution of diltiazem with pregnancy may be more apparent than real. Diltiazem was able to distribute readily into the foetal compartment while there was some delay in the foetal distribution of desacetyldiltiazem.

The work presented here strongly supports previous evidence that the actions of diltiazem *in vivo* on the uterus are due to the parent drug and that the metabolite desacetyldiltiazem does not significantly contribute to the observed biological effects. There was marked inhibition of uterine contractions after bolus administration of diltiazem, when desacetyldiltiazem was rarely detected. Significant inhibition of uterine contractions was observed in all rats receiving an infusion of diltiazem, yet 5/13 animals showed no detectable levels of desacetyldiltiazem. Furthermore, a return to control values of uterine contractions was noted concomitant with declining serum diltiazem concentrations after cessation of diltiazem infusion, even though serum desacetyldiltiazem concentrations remained elevated. Finally, although desacetyldiltiazem exhibited inhibitory activity on the rat isolated uterus, it was 3.2 fold less potent than diltiazem. Similar, but less strongly supported arguments, apply to the effects of diltiazem on cardiovascular parameters.

The work presented here also supports the suggestion that the inhibitory effect of diltiazem *in vivo* is due to a direct action on the myometrium and is not produced by some indirect mechanism (Abel & Hollingsworth, 1985; 1986a,b). It has previously been shown that the rank order of potencies of several calcium entry blockers *in vivo* (Abel & Hollingsworth, 1985) was identical to that observed against spontaneous contractions of the rat isolated uterus (Granger *et al.*, 1985). It has now been shown that the IC_{50} *in vivo* (0.5 $\mu g\,ml^{-1}$) is similar to the IC_{50} on the isolated uterus (0.79 $\mu g\,ml^{-1}$) against 40 mM K^+. We therefore conclude that the inhibition of uterine contractions observed *in vivo* after diltiazem administration is due to the parent drug acting directly on the myometrium.

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