International Journal of Pharmaceutics, 64 (1990) 171–180
Elsevier

IJP 02169

Drug lipophilicity and release pattern of some β-blocking agents after intra-adipose injection in pigs

F. Kadir [1], J. Zuidema [1], A. Pijpers [2], A. Vulto [3] and J.H.M. Verheijden [2]

[1] *Department of Pharmaceutics,* [2] *Department of Herd, Health and Reproduction, and* [3] *Department of Veterinary Pharmacology, Pharmacy and Toxicology, University of Utrecht, Croesestraat 79, 3522 AD Utrecht (The Netherlands)*

(Received 1 February 1990)
(Accepted 10 April 1990)

Key words: Intra-adipose injection; β-Blocking agent; Drug lipophilicity; Bioavailability; Fat distribution

Summary

Release rates from intramuscular and intra-adipose injection sites have turned out to be dependent upon several factors including injection depth. Little is known about the interaction between drug lipophilicity and transport rate of drugs through adipose and muscle tissue. The principal objective of the present study was to determine to what extent drug lipophilicity affects release and release rate from adipose tissue. Nine pigs were given intravenous (0.1 mg/kg), intramuscular (0.2 mg/kg) and intra-adipose (0.2 mg/kg) injections of propranolol, alprenolol, carazolol, metoprolol and atenolol. The fraction not-absorbed vs time plots after intramuscular and intra-adipose injection showed a biphasic decline for all model compounds with the exception of atenolol being the most hydrophilic drug. This biphasic decline indicates that two different mechanisms may be involved in drug release. Initial release rates after intra-adipose injection were negatively correlated (Kendall's rank order test) with fat-buffer partition constants. The second release phase was best characterized by the extent of 24 h release. The amounts (mean ± S.D.) released after 24 h for propranolol, alprenolol, carazolol, metoprolol and atenolol are 42 ± 15, 38 ± 9, 45 ± 18, 48 ± 12 and 99 ± 12% following intra-adipose injection and 57 ± 8, 36 ± 18, 38 ± 15, 55 ± 14 and 104 ± 14% after intramuscular injection, respectively. Incomplete release at 24 h can be explained by the sunk solvent drag after absorption of the solvent is complete. Octanol-buffer partition and pig-fat-buffer distribution constants turned out to be positively correlated.

Introduction

Intramuscular (i.m.) and subcutaneous (s.c.) injections are important routes of drug administration and can be used to provide a sustained release of the active ingredient. Release rates from these injection sites have appeared to be dependent upon several factors such as body movement (Evans et al., 1975; Mundie et al., 1988), injection site (Wise and Reeves, 1974), sex, molecular size (Ballard et al., 1968), pK_a (Kurosaki et al., 1988), drug solubility (Hirano et al., 1981), injection volume, injection depth, initial concentration, blood supply at the injection site (Evans et al., 1975) and injection technique (Brumback et al., 1982; Hornig and Dorndorf, 1983; Laube et al., 1988).

Recently, the available literature on injection depth has been reviewed and a number of studies, case reports and mechanisms of release and absorption have been discussed (Zuidema et al.,

Correspondence: F. Kadir, Department of Pharmaceutics, University of Utrecht, Croesestraat 79, 3522 AD Utrecht, The Netherlands.

1988). Many of the failures of parenteral drug treatment and prophylaxis mentioned in this review could be explained by too shallow an injection. Drugs which were meant to be given intramuscularly were probably deposited in the subcutaneous adipose fat layer, which is supposed to have stronger retarding effects on drug release than muscle tissue. An unexpected retardation of drug release will result in low drug levels which may lead to reduced efficacy of treatment. Too shallow an injection has turned out to be of considerable risk especially in the gluteal region in women (Modderman et al., 1982, 1983). This explains the often observed sex differences in intramuscular drug absorption rate (Pieters et al., 1986).

Morrison (1982) was the first to use the term 'intra-adipose' to describe the intended injection in the subcutaneous adipose layer. In accordance with his suggestion, this term (henceforth referred to as i.ad.) will be used throughout this article.

From the review mentioned above (Zuidema et al., 1988) it appeared that insufficient knowledge is available on the interaction between drug lipophilicity and transport rate of drugs through adipose or muscular tissue.

The principal objective of this study was therefore to investigate to what extent drug lipophilicity affects release and release rate from adipose and muscular tissue.

In addition, it was considered interesting to establish whether the partition characteristics of the test drugs between adipose tissue and water could effectively be described by their octanol-water partition constants. This possibly enables one to relate the release properties of a drug to its documented octanol-water partition coefficient.

As pig adipose tissue resembles that in humans both chemically and physiologically, the pig was chosen as an animal model. The following β-blocking agents were used as test drugs: propranolol, alprenolol, carazolol, metoprolol and atenolol in order of decreasing lipophilicity. These drugs are an ideal group of model compounds, since they differ markedly in lipophilicity but only slightly in pK_a (see Table 2).

Carazolol is a β-blocking agent which is used for the treatment and prevention of complications resulting from stress in pigs. With regard to residue problems in meat it is an interesting drug in veterinary practice (Ballarini and Guizardi, 1981; Bostedt and Rudloff, 1983).

Materials and Methods

Injection solutions

Aqueous solutions of propranolol, alprenolol, metoprolol and atenolol were prepared by dissolving propranolol and alprenolol as their hydrochloride salts (gifts from ICI-Farma, Rotterdam, The Netherlands), metoprolol as the tartrate salt and atenolol as the basic compound (both gifts from Astra Pharmaceutica B.V., The Netherlands), respectively. These injection solutions contained 20 mg of the corresponding parent compound per ml. NaCl was added to isotonicity, the pH being adjusted to 7.4 with HCl or NaOH solutions (1.0 N) if necessary. The solutions were sterilised by autoclaving (15 min at 121°C). Drug contents were determined after sterilisation and deviated by less than 5% from the declared amount.

Animals

The experiments were performed with nine healthy castrated conventional pigs from the University's breeding farm (Great Yorkshire × Dutch Landrace, F1-hybrids; body weight: 96–116 kg). The pigs were placed in individual metabolic pens provided with facilities for drinking (ad libitum) and eating (twice daily 0.75 kg pelleted and antibiotic-free food, Eemstroom-Leusden, The Netherlands).

In order to facilitate blood collection during the experiment, an indwelling catheter was placed in the external jugular vein. Surgery was performed 2 days before the start of the experiment. The catheter was fixed at the rear of the neck in order to be freely accessible for sampling (Pijpers et al., 1989).

Injection technique / dosage

The thickness of the subcutaneous fat layer at the injection site (dorsal neck region) was measured using a Digital Back Fat Indicator (RencoLean-Meater®, Renco Corp., Minneapolis) and

injection sites were marked. Fat layer thicknesses ranged from 19 to 28 mm (mean ± S.D. = 21 ± 3 days). After determination of the thickness of the fat layer, the drugs (with the exception of carazolol) were administered alternately on the left and right dorsal neck region: intra-adiposely (i.ad.), 0.2 mg/kg; intramuscularly (i.m.), 0.2 mg/kg; or intravenously (i.v.), 0.1 mg/kg. Intravenous injections (0.1 mg/kg) were given via the inserted cannula. For carazolol the procedures were similar, however with different dosages.

Depending upon the body weight of the individual pig, volumes varying from 0.96 to 1.16 ml corresponding with dosages of 0.2 mg/kg were administered i.ad. and i.m. Carazolol was administered i.v., i.m. and i.ad. in a dose of 0.025 mg/kg (5 ml) as the commercially available solution Suacron® (gift from Upjohn Veterinary Division, Ede, The Netherlands).

Intra-adipose injections were given using either a 25-gauge 5/8 0.5 × 16 (B-D®, Microlance) or a 26-gauge 1/2 0.45 × 13 (Jecton-S) needle depending upon the thickness of the fat layer as measured before injection. Intramuscular injections were administered with an 18-gauge 3/2 1.2 × 40 (Braun, Sterican®) needle. Intravenous administrations (0.1 mg/kg) were given via the inserted cannula.

Intra-adipose and intramuscular injections were applied at an angle of 45 and 90°, respectively, and immediately after injection it was checked whether solution leaked back along the track of the needle.

Study design

The study was conducted with two ($n = 5$ and $n = 4$) groups of pigs. One group received three and the other group received two test drugs in a cross-over design, each drug being administered i.v., i.m. and i.ad. Five pigs (randomly assigned) received propranolol, alprenolol and carazolol while the remaining four received metoprolol and atenolol (wash-out period: 1 day).

Blood samples, 5 ml each time, were drawn at regular times beforehand and at 10, 20, 30, 45 and 60 min and 2, 3, 4, 6, 8, 10 and 24 h after injection. Blood was allowed to clot for about 45 min and centrifuged (15 min, 2000 × g). Serum was transferred to a glass tube and stored at −20 °C until analysis.

Analysis of blood samples

Drug concentrations of propranolol, alprenolol and metoprolol in serum were determined using the HPLC method with fluorimetric detection according to Duchateau (1986). Atenolol concentrations in serum were determined as described by Gengo et al. (1989). Carazolol concentration in serum was determined using a reversed-phase HPLC method developed for this study (Kadir and Zuidema, 1990).

Determination of partition coefficients

The distribution of the drugs between adipose fat and an isotonic phosphate buffer solution (pH = 7.4) was determined on freshly excised and homogenized (using an Ultra-Turrax mixer) adipose tissue. Partition constants between octanol and phosphate buffer were determined using the shake-flask method (Schurman and Turner, 1978; Dunn et al., 1986).

Pharmacokinetic and statistical analysis

The elimination rate constant (k_{el}) was determined by fitting the individual data from the terminal part of the intravenous drug concentration in serum-time profiles to the log-linear regression analysis. Maximum concentrations, C_{max}, were read directly from the drug concentration in serum vs time data. Distribution volumes (V_d) were calculated by dividing the absolute amount of drug administered (i.v.) by the extrapolated concentration for $t = 0$. Clearance values were obtained by multiplication of k_{el} and V_d. AUC values were calculated by the linear trapezoidal rule. Extrapolation to infinity was performed, where possible, using the last measured serum concentrations divided by the corresponding intravenous k_{el} values.

The fraction non-absorbed data were calculated from the cumulative AUC per time point divided by the corresponding intravenous AUC until infinity and corrected for differences in dose. The release rate (rr) constant was calculated from these plots by log-linear regression analysis over the first linear part of the curve.

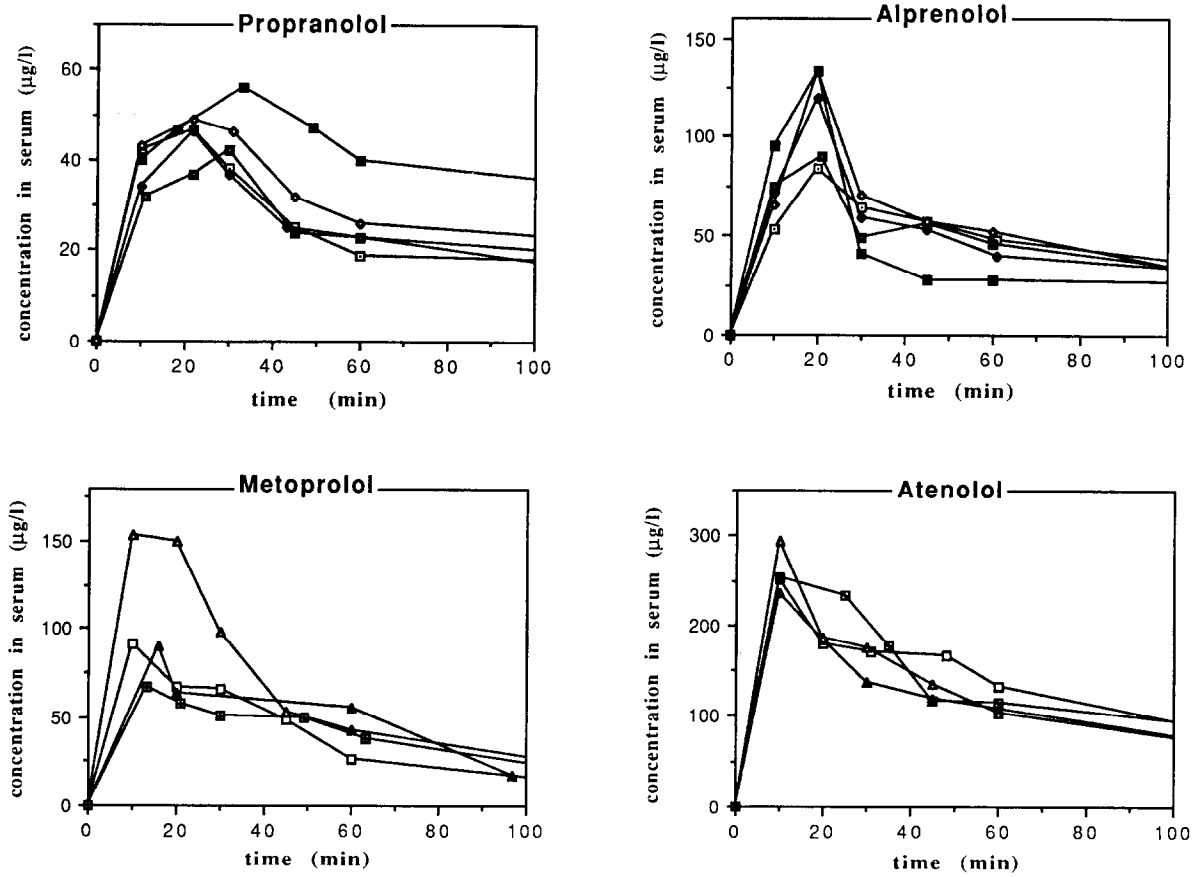

Fig. 1. Individual serum concentration-time profiles following intra-adipose injection (0.2 mg/kg) of propranolol, alprenolol, metoprolol and atenolol.

The concentration-time curves are individually presented in order to avoid false characteristics (Zuidema and Wynne, 1989). This was not necessary for the fraction not-absorbed plots. These are presented as mean plots. Data were considered to be normally distributed when the mean was greater

TABLE 1

Kinetic parameters (mean ± S.D.) following intravenous administration and the 24 h release after intramuscular ($FR^{i.m.}$) and intra-adipose ($FR^{i.ad.}$) administration

	k_{el} (h^{-1})	V_d ($l\,kg^{-1}$)	Cl ($l\,h^{-1}\,kg^{-1}$)	$FR_{i.ad.}$	$FR_{i.m.}$
Propranolol	0.31 ± 0.08	2.91 ± 0.58	0.90 ± 0.17	42 ± 15	57 ± 8
Alprenolol	0.72 ± 0.16	1.45 ± 0.47	0.99 ± 0.24	38 ± 9	36 ± 18
Carazolol	0.37 ± 0.16	0.45 ± 0.16	0.16 ± 0.07	45 ± 18	38 ± 15
Metoprolol	0.41 ± 0.07	2.60 ± 0.71	1.05 ± 0.38	48 ± 12	55 ± 14
Atenolol	0.28 ± 0.08	1.95 ± 0.30	0.54 ± 0.13	99 ± 12	104 ± 14

Cl, total clearance; k_{el}, elimination constant; V_d, distribution volume.

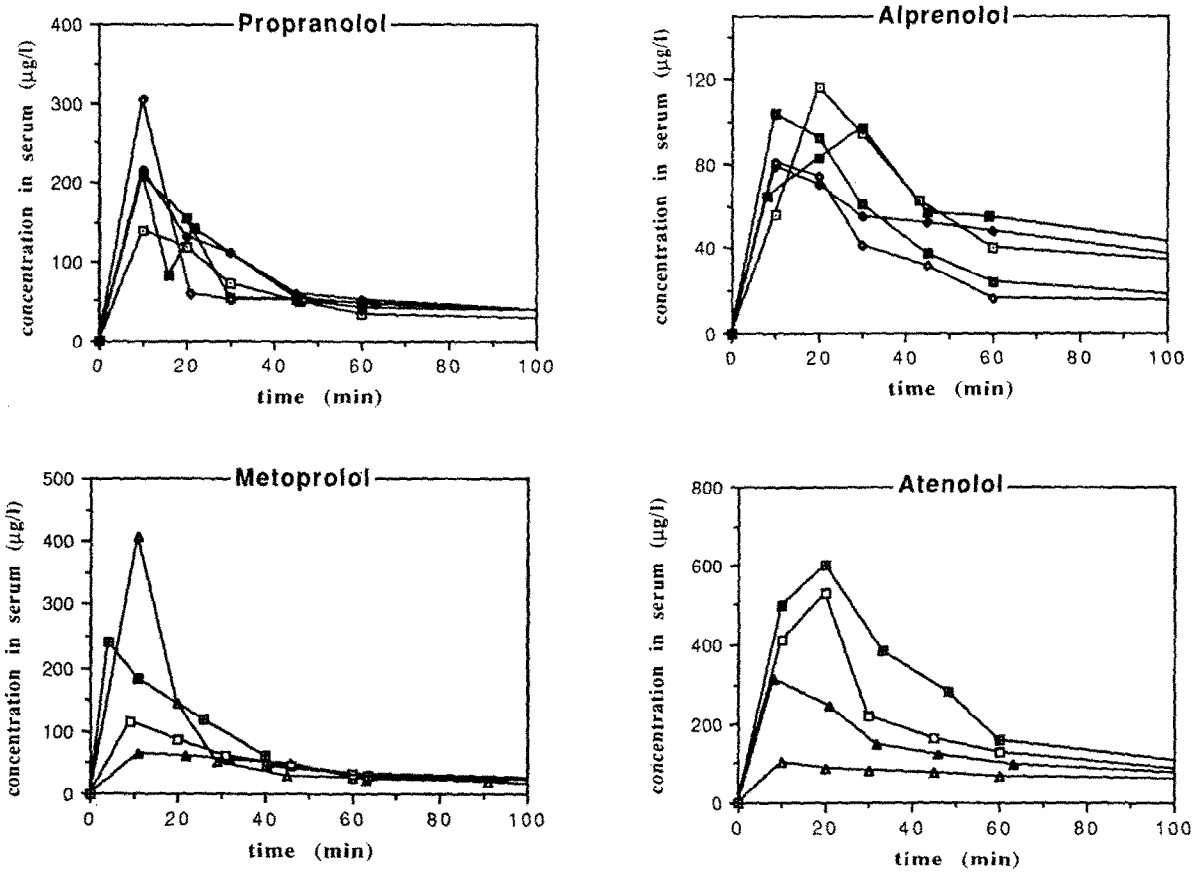

Fig. 2. Serum concentration-time profiles following intramuscular injection (0.2 mg/kg) of propranolol, alprenolol, metoprolol and atenolol.

than 2-times the standard deviation of the data set and no indications of deviant distributions existed. Standard deviations are given as a dispersion measure. For difference testing, paired and non-paired t-tests and for correlation the Kendall's rank order test have been applied. The level of significance was chosen at $\alpha = 0.05$.

Results

Individual drug profiles in serum following intra-adipose and intramuscular administrations are shown in Figs 1 and 2, respectively.

The pharmacokinetic parameters derived from the intravenous administrations and the extent of 24 h release after intramuscular and intra-adipose administrations are summarized in Table 1. Results are expressed as mean ± S.D.

Table 2 summarizes the pK_a values, release rates (rr), pig-fat-buffer distribution constants and

TABLE 2

Pig-fat-buffer distribution constants, pK_a, release rates (rr) and C_{max} (mean ± S.D.) values after intra-adipose injections of the test drugs

	pK_a	P_{fat}	rr ± S.D. (min^{-1})	C_{max} ± S.D. ($\mu g\ l^{-1}$)
Propranolol	9.5	3.80	0.0011 ± 0.0002	47 ± 5
Alprenolol	9.7	2.15	0.0017 ± 0.0005	112 ± 24
Carazolol	9.5	2.02	0.0025 ± 0.0010	161 ± 54
Metoprolol	9.7	0.65	0.0041 ± 0.0010	100 ± 37
Atenolol	9.6	0.03	0.0043 ± 0.0008	259 ± 26

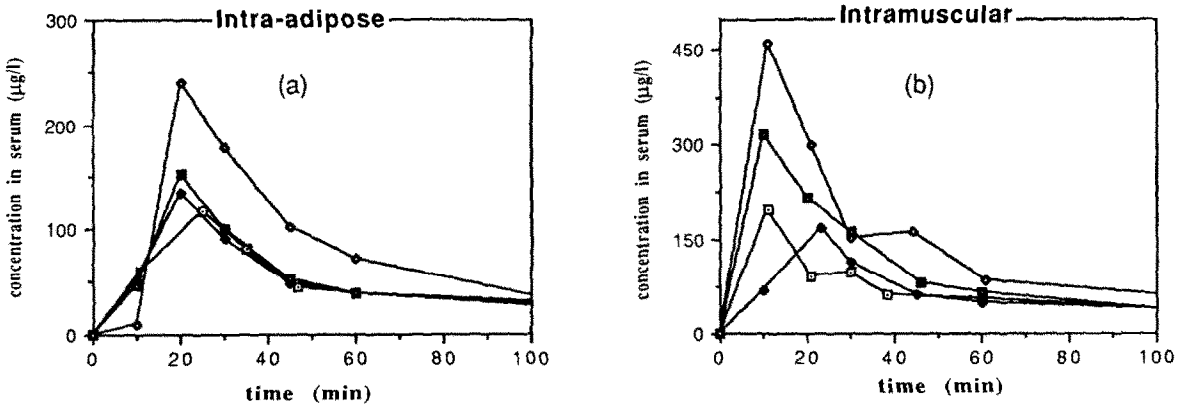

Fig. 3. Serum concentration-time profiles following intra-adipose (a) and intramuscular (b) administration (0.025 mg/kg) of carazolol.

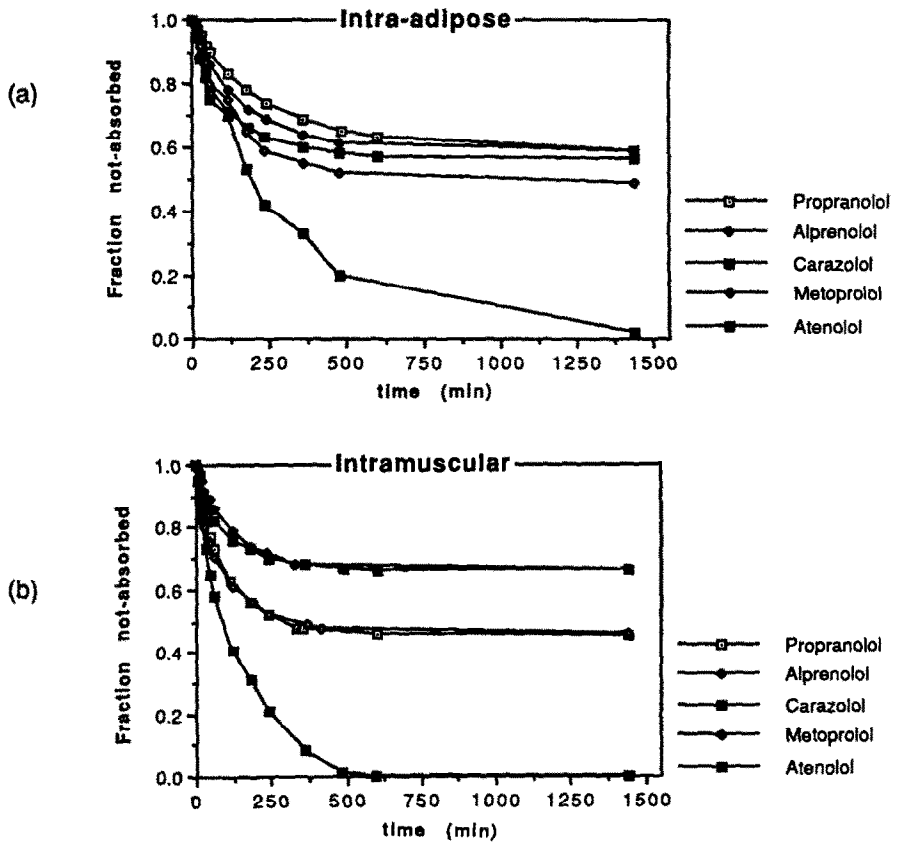

Fig. 4. Fraction not-absorbed drug vs time after i.ad. (a) and i.m. (b) administration.

C_{max} values of the test drugs following intra-adipose administration. Results are expressed as mean ± S.D.

The carazolol concentration in serum vs time curves after intramuscular and intra-adipose administration are presented in Fig. 3.

The fraction not-absorbed plots of the five test drugs 24 h after intramuscular and intra-adipose administration are shown in Fig. 4a and b, respectively. Both sets of the fraction not-absorbed curves (i.e. intramuscular and intra-adipose) of all model compounds with the exception of atenolol show a biphasic decline: a rapid phase followed by a very slow release.

The rate of the initial release phase can be characterized by its slope over the first linear part. These slopes represent release rates. Fig. 5 shows the relation between release rate (reflected by the slope of the initial phase) and drug lipophilicity. By performing a non-parametric rank correlation test (Kendall) on these data a significant negative correlation could be established.

The second phase is the best characterized by its 24 h release. It turned out that the absorption was incomplete at 24 h for all drugs with the exception of the hydrophilic atenolol after both intramuscular and intra-adipose administration. The released amounts for propranolol, alprenolol, carazolol, metoprolol and atenolol were 42 ± 15, 38 ± 9, 45 ± 18, 48 ± 12 and 99 ± 12% following intra-adipose injection and 57 ± 8, 36 ± 18, 38 ± 15, 55 ± 14 and 104 ± 14% after intramuscular injection. The difference in extent of release at 24 h between the more lipophilic drugs propranolol, alprenolol, carazolol and metoprolol was not statistically significant. However, differences between these test agents and atenolol did result in statistically significant differences after both intramuscular and intra-adipose injections (paired and non-paired t-tests).

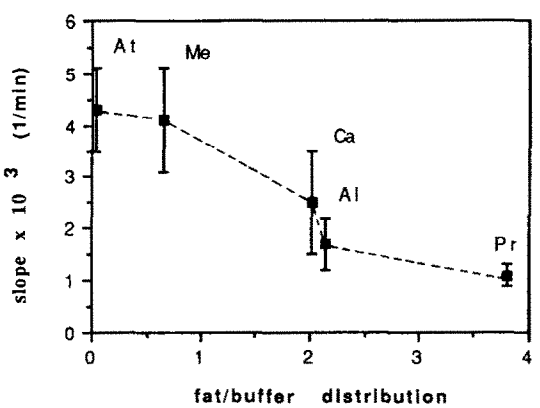

Fig. 5. Correlation between the fat-buffer distribution constants and release rates (reflected by the slope) on intra-adipose administration of propranolol (Pr), alprenolol (Al), carazolol (Ca), metoprolol (Me) and atenolol (At).

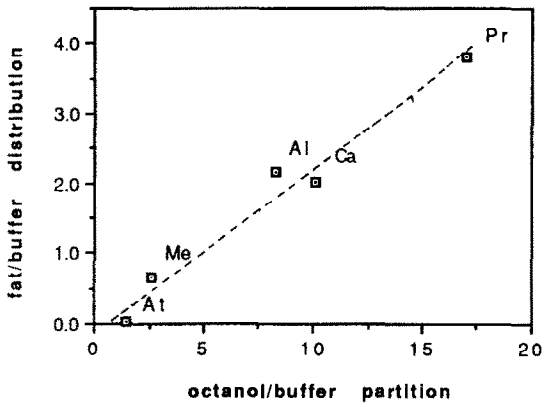

Fig. 6. Correlation between the octanol-buffer partition and pig-fat-buffer distribution coefficients of the β-blocking agents.

Following intramuscular administration the variation in C_{max} values was higher than those observed on intra-adipose injection. Coefficients of variation following intramuscular and intra-adipose injection were 28, 18, 47, 80 and 59% and 11, 21, 34, 37 and 10% for propranolol, alprenolol, carazolol, metoprolol and atenolol, respectively.

Fig. 6 shows the relation between fat-buffer distribution and octanol-buffer partition coefficients.

Discussion

The biphasic decline of the fraction not-absorbed curves suggests that at least two different mechanisms are involved in the release of the intra-adipose and intramuscular depots.

With regard to the initial release rate values, significant differences between the test drugs on

intra-adipose injection were observed. Fig. 5 demonstrates that these initial release rates after intra-adipose injection are negatively correlated (Kendall's rank order test) with fat-buffer partition constants. The release rates are higher and more variable after intramuscular than after intra-adipose administration.

These results can be explained by assuming that fat-containing components of the tissue have stronger retarding effects on more lipophilic drugs. One can imagine that this is caused by a difference in diffusion rate through the layers, which may behave like some kind of chromatographic system. Moderately hydrophilic substances are mainly transported through the intercellular aqueous media whereas the transcellular route is more important for lipophilic substances. As adipose blood supply is localized in the interconnective fibrous tissue surrounding and interconnecting the adipocytes, hydrophilic drugs are supposed to reach the capillary vessels sooner than lipophilic compounds. This hypothesis is supported by a recent study of Artursson and Karlsson (1990) on the relative contributions of the transcellular and paracellular pathways to the overall in vitro intestinal transport of a series of β-blocking agents. From this study, it appeared that non-cellular resistance increased with increasing lipophilicity while cellular resistance was high at low lipophilicity.

The slope of the second phase within the observation period is too small to be calculated with sufficient accuracy from the few observation points. Therefore, the second release phase in this experiment is best characterized by the extent of 24 h release. The extent of 24 h release after intra-adipose administration turned out to be dependent upon the lipophilicity of the drug. More lipophilic drugs such as propranolol, carazolol, alprenolol and, to a lesser extent, metoprolol show incomplete release at 24 h, whereas only the most hydrophilic drug, atenolol, is absorbed quickly and completely after both i.ad. and i.m. injection. The incomplete 24 h release of carazolol has also been observed in a study with intramuscular injections of radiolabeled carazolol (Rudloff, 1982).

Incomplete release at 24 h or a slow second phase release can be explained either by the sunk solvent drag after the absorption of the solvent components is complete, or by the supposition of binding occurring at the injection sites and subsequently a very slow release, or by tissue metabolism at the injection site.

The first explanation of the changing solvent drag is self-evident and the most likely. The very hydrophilic atenolol is then absorbed at a rate about the same as or even higher than that of the solvent components. This explanation has been adopted in the literature in a study with intramuscular phenytoin (Kostenbauder et al., 1976).

With regard to the second possibility, protein components within muscle tissue itself as well as components from intercellular spaces could be involved in protein binding.

The third possible explanation for the incomplete 24 h release after intramuscular administration might be that the model compounds are metabolized at different rates by rapid tissue metabolism. However, there is no evidence in the literature to support this hypothesis.

The greater variation in C_{max} after intramuscular administration might be explained by the observed variation in body movement between the animals, since absorption at the intramuscular injection site is susceptible to changes in blood flow (Zuidema et al., 1988). Although the metabolic pens in which the animals were fixed were quite small, movement was still possible and increased as the pigs became familiar with the experimental conditions. Furthermore, tissue sites at which the drug is actually deposited should also be considered. Injections into muscular tissue can be either intramuscular or intermuscular, resulting in different absorption rates (Groothuis et al., 1980; Marshall and Palmer, 1980).

It should be mentioned that although the utmost care was taken with the intra-adipose and intramuscular injection techniques, the possibility that some injections did not reach the target tissue should be taken into consideration as well. Nevertheless, a number of conclusions can be drawn from the present study.

The release rate following intra-adipose and intramuscular injection is biphasic in general.

The initial release rate is dependent on the drug lipophilicity, after intra-adipose injection being lower for more lipophilic drugs.

The 24 h release after both intramuscular and intra-adipose injection is incomplete for lipophilic β-blocking agents, indicating a slow second release phase.

Injection depth and thickness of subcutaneous fat layers should be taken into account carefully, especially when highly lipophilic drugs are injected. Intramuscularly injected drugs are absorbed with both rate and extent varying over a considerable range of values. After intra-adipose injection drug absorption is slower but shows less variation.

Octanol-buffer partition and pig-fat-buffer distribution constants have turned out to be positively correlated, which enables one to predict drug release by means of its partition constants.

References

Artursson, P. and Karlsson, J., Passive absorption of drugs in CACO-2 cells. In Wilson, G., Illum, L. and Davis, S.S. (Eds.), *Pharmaceutical Applications of Cell and Tissue Culture,* Plenum, New York, 1990, in press.

Ballard, B.E., Biopharmaceutical considerations in subcutaneous and intramuscular drug administration. *J. Pharm. Sci.*, 57 (1968) 357–378.

Ballarini, G. and Guizardi, F., Wirkung des β-blockers carazolol bei Transportkrankheit oder stress-Syndrom des Schweines. *Tierärztliche Umschau,* 36 (1981) 171–180.

Bostedt, H. and Rudloff, P.R., Prophylactic administration of the beta-blocker carazolol to influence the duration of parturition in sows. *Theriongenology,* 20 (1983) 191–196.

Brumback, R.A., Empting, L., Susag, E. and Staton, D., Muscle fibrosis associated with intramuscular chlorpromazine adminstration. A preliminary report. *J. Pharm. Pharmacol.,* 34 (1982) 526–528.

Duchateau, G.S.M.J.E., Studies on nasal drug delivery. Thesis, Amsterdam, 1986.

Dunn, W.J., III, Block, J.H. and Pearlman, R.S., *Partition Coefficients: Determination and Estimation,* Pergamon, New York, 1986.

Evans, E.F., Proctor, J.D., Fratkin, M.J., Velandia, J. and Wasserman, A.J., Blood flow in muscle groups and drug absorption. *Clin. Pharmacol. Ther.,* 17 (1975) 44–47.

Gengo, F.M., Fagan, S.C., Hopkins, L.N., Wagner, D. and Schuster, D.P., Nonlinear distribution of atenolol between plasma and cerebrospinal fluid. *Pharm. Res.*, 3 (1989) 248–251.

Groothuis, D.G., Werdler, M.E.B., Van Miert, A.S.J.P.A.M. and Van Duin, C.T.M., Factors influencing the absorption of ampicillin administered intramuscularly in dwarf goats. *Res. Vet. Sci.,* 29 (1980) 116–117.

Hirano, K., Ichihashi, T. and Yamada, H., Studies on the absorption of practically water-insoluble drugs following injection. II. Intramuscular absorption from aqueous suspensions in rats. *Chem. Pharm. Bull.,* 29 (1981) 817–882.

Hornig, C. and Dorndorf, W., Kombinierte Nervenschäden und Embolia cutis medicamentosa nach intraglutäaler Fehlinjektion. *Dtsch. Med. Wschr.,* 108 (1983) 221–223.

Kadir, F. and Zuidema, J., A high performance liquid chromatographic method for the determination of carazolol in serum or plasma of pigs. *J. Chromatogr. Biomed. Appl.,* 527 (1990) 461–466.

Kostenbauder, H.B., Rapp, R.P., McGovren, J.P., Foster, T.S., Perrier, D.G., Blacker, H.M., Hulon, W.C. and Kinkel, A.W., Bioavailability and single dose pharmacokinetics of intramuscular phenytoin. *Clin. Pharmacol. Ther.,* 18 (1976) 449–455.

Kurosaki, Y., Takatori, T., Kitayama, M., Nakayama, T. and Kimura, T., Application of propranolol to the keratinized oral mucosa: avoidance of first pass elimination and the use of 1-Dodecylazacycloheptan-2-one (azone) as an absorption enhancer of bioadhesive film-dosage form. *J. Pharmacobio-Dyn.*, 11 (1988) 824–832.

Laube, H., Pinter, W. and Federlin, K., Investigation of the prolonged hypoglycemic effect of human proinsulin in diabetes mellitus. *Horm. Metab. Res.,* 18 (1988) 12–15.

Marshall, A.B. and Palmer, G.H., Injection sites and drug bioavailability. *Trends in Veterinary Pharmacology and Toxicology* (proceedings of the first European Congress on Veterinary Pharmacology and Toxicology 1979, Zeist, The Netherlands), 1980, pp. 54–60, Elsevier, Amsterdam.

Modderman, E.S.M., Hilbers, H.W., Zuidema, J. and Merkus, F.W.H.M., Injections into fat instead of muscle. *N. Engl. J. Med.*, 307 (1982) 1581.

Modderman, E.S.M., Merkus, F.W.H.M., Zuidema, J., Hilbers, H.W. and Warndorff, T.T., Sex differences in the absorption of dapsone after intramuscular injection. *Int. J. Lepr.* 51 (1983) 359–365.

Morrison, M.C., Jr., Injections into fat instead of muscle. *N. Engl. J. Med.,* 307 (1982) 1580.

Mundie, T.G., Pamplin, C.L., III, Phillips, Y.Y. and Smallridge, R.C., Effect of exercise in sheep on the absorption of intramuscular atropine sulfate. *Pharmacology,* 37 (1988) 132–136.

Pieters, F.A.J.M., Zuidema, J. and Merkus, F.W.H.M., Sustained release properties of an intra-adiposely administered dapsone depot injection. *Int. J. Lepr.,* 54 (1986) 383–387.

Pijpers, A., Noordhuizen-Stassen, E.N., Goedegebuure, S.A., Van Dobbenburgh, O.A., Roosendaal, M., Cornelissen, A.H.M. and Verheijden, J.H.M., Intravenous catheterization of conventional pigs without application of antimicrobial agents. *Vet. Q.,* 11 (1989) 216–221.

Rudloff, P., Investigation into the blood kinetics of 14-C-carazolol. The residual quantities of this substance in various tissues and its excretion in the pig. Praemix Wirkstoff, Mannheim, F.R.G., report 1982.

Schurman, W. and Turner, P., A membrane model of the human oral mucosa as derived from buccal absorption performance and physicochemical properties of the β-blocking drugs atenolol and propranolol. *J. Pharm. Pharmacol.*, 30 (1978) 137–147.

Wise, R. and Reeves, D.S., Pharmacological studies on cephacetrile in human volunteers. *Curr. Med. Opin.*, 2 (1974) 249–255.

Zuidema, J., Pieters, F.A.J.M. and Duchateau, G.S.M.J.E., Release and absorption rate aspects of intramuscularly injected pharmaceuticals. *Int. J. Pharm.*, 47 (1988) 1–12.

Zuidema, J. and Wynne, H.J.A., Data reduction problems in biopharmaceutics and pharmacokinetics. *Pharm. Weekbl. Sci. Ed.* 6 (1989) 787–793.