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Absorption of exaprolol from the in-situ gastrointestinal tract of rats and dogs

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The absorption rate of the β-adrenoceptor blocking drug, exaprolol, from the gastrointestinal tract was studied using in-situ methods in the rat and dog. Exaprolol was rapidly absorbed from the small and large intestine of rats and from the ileum of dogs. The cardiac output and regional blood flow decreased in rats to approximately one half of the original values within 30 min of the in-situ experiment. The logarithm of the amount vs time plots from dogs were linear, whereas with rats a curvilinearity appeared apparently because of the blood flow-limited absorption kinetics of this highly lipophilic drug. The data obtained suggest that exaprolol is suitable for administration in sustained release form.

Exaprolol, 1-(2-cyclohexylphenoxy)-3-isopropylamino-2-propanol (Carissimi et al 1976; Jendrichovský et al 1978), is another member of the series of β-adrenoceptor blocking drugs having a cycloalkyl group in the *ortho*-position of the aromatic ring. The cyclopentyl derivative has broad clinical application (Vierhapper et al 1980) and the cyclopropyl (Boissier et al 1971) and cyclohexyl (Trnovec et al 1982: Tomčíková et al 1984; Hughes et al 1984) derivatives are being investigated. The rapid appearance of exaprolol in plasma after oral administration indicates that almost 100% of the drug is absorbed from the upper part of the small intestine (Trnovec et al 1982). A prolonged action of exaprolol is desirable and for a sustained-release formulation, absorption of the drug from lower segments of the gastrointestinal tract is essential.

In this study, in rats, the absorption of exaprolol from various parts of the small and large intestine was investigated by an in-situ method. In dogs, the chronic intestinal loop model was used for determination of the exaprolol absorption rate. It has been shown recently (Taylor et al 1981) that in the in-situ rat gut technique, the in-vivo absorption rates may be affected by the decreasing intestinal blood flow following surgical manipulation and anaesthesia. The blood flow rate through the intestinal villus can be a rate-limiting 'barrier' to drug absorption, particularly to the absorption of drugs that rapidly penetrate other mucosal barriers (Hayton 1980). So far, no quantitative data have been published about the state of the intestinal perfusion in the in-situ intestinal loop preparation in the course of the experiment. Therefore, the present study was completed by parallel measurements of the regional intestinal blood supply and cardiac output using the microsphere method.

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Methods

Absorption measurements in rats. The rate of absorption of exaprolol from the GIT of rats was assessed by the method of Doluisio et al (1969) modified by Taylor & Grundy (1980). In this method the rate of drug disappearance from an in-situ cannulated segment of the rat gut is used as a measure of absorption. Male Wistar rats (230–250 g) were fasted 16 h before the experiment but had free access to water. Anaesthesia was induced and maintained by inhalation of halothane (Narcotan, Léčiva, n.p., Praha, Czechoslovakia). After dissection, the desired region of the GIT was cannulated at both ends by polyethylene cannulae (5 cm length, 0·25 cm i.d.). The following segments of the GIT were investigated: stomach, duodenum, jejunum and ileum, caecum and colon. The contents of the cannulated segments were first washed out by perfusion with saline (0·9% NaCl) solution (37 °C). The solution of tritium-labelled exaprolol (0·2 mg ml^{-1}; 16·6 kBq ml^{-1}) together with a nonabsorbable marker [^{14}C]polyethylene glycol-4000 (PEG) (0·002 mg ml^{-1}) (Amersham International plc) was introduced through the proximal cannula. The temperature of the solution was 37 °C and its volume ranged from 2–5 ml depending on the size of the examined segment of GIT. Samples of 0·1ml were taken immediately after drug introduction (t = 0) and at 5, 10, 15, 30, 45 and 60 min intervals.

Blood flow measurements in rats. Regional intestinal blood supply was investigated in rats treated in the same manner as above except that no radiolabelled drug was introduced into the segment. Perfusion only of the upper third of the small intestine was performed. A polyethylene catheter was inserted into each femoral artery and blood pressure was monitored continuously from the right femoral artery. The catheter in the left femoral artery was connected to a suction pump for taking reference blood samples during the microsphere injections. A catheter was inserted into the left ventricle of the heart through the right carotid artery and was used for microsphere injections. Carbonized micro-

spheres labelled with ^{85}Sr and ^{46}Sc measuring 15 ± 5 μm (3M Co, St Paul, Minn., USA) in saline were used. Immediately before the injection they were sonicated and mechanically agitated. Approximately 300 000–400 000 microspheres in a volume of 80–100 μl were injected each time. A reference blood sample was drawn at a constant rate of 0·5 ml min^{-1}. The insertion of catheters was followed by the absorption study. The first microsphere injection was given about 1 h after induction of anaesthesia (after completing the surgical procedure) and the second one 30 min later. The rats were killed and the liver, stomach, small intestine and large intestine were removed. The small intestine was divided into three segments. Tissue samples were weighed and the radioactivity of each nuclide determined in a gamma-spectrometer. From the data obtained, the blood flow and cardiac output were calculated by the method of McDevitt & Nies (1976). The values presented are means ± s.e.m. Comparisons were made by means of Student's *t*-test for paired observations.

Absorption measurements in dogs. Male mongrel dogs (10–12 kg) were used in the absorption study. Thiry-Vella ileal loops were surgically constructed in each dog under aseptic conditions using a procedure described by Markowitz et al (1964) and modified by Taylor et al (1981). Approximately 30 ml of drug solution in unbuffered saline were introduced into the loop. The absorption rate was investigated at two concentrations of the drug. The solution contained either 0·33 or 0·033 mg ml^{-1} of exaprolol (1·66 kBq ml^{-1}) and nonabsorbable marker [^{14}C]PEG in a concentration 0·62 μg ml^{-1} (0·5 kBq ml^{-1}). The following procedure was analogous to that used for absorption measurements in rats. Bray's scintillation solution (10 ml) was added to the samples of 0·5 ml. The ^{3}H and ^{14}C radioactivity of samples was determined by a liquid scintillation spectrometer (Packard TriCarb 300 C).

The fractional amount of drug in the sample was calculated from the equation,

$$f = \frac{[^{3}H]EXA_t}{[^{3}H]EXA_0} \cdot \frac{[^{14}C]PEG_0}{[^{14}C]PEG_t}$$

where f is the fraction of the initial amount of the drug remaining in the segment, $[^{3}H]EXA_0$ and $[^{14}C]PEG_0$ is the radioactivity of [^{3}H]exaprolol and [^{14}C]polyethylene glycol in the sample at the time t = 0, respectively, and $[^{3}H]EXA_t$ and $[^{14}C]PEG_t$ is the radioactivity of [^{3}H]exaprolol and [^{14}C]polyethylene glycol at the time t, respectively.

The logarithm of the amount vs time plots as a whole or their initial linear portions were fitted by exponential equations by least square method. The first order disappearance rate constants and the corresponding half-lives were calculated from the slopes.

Results

The exaprolol disappearance plots for the rat gastrointestinal tract segments are shown in Fig. 1 and the corresponding rate constants and half-lives of absorption in Table 1. The decrease of the logarithm of the amount of exaprolol in the lumen of the rat stomach was slow and linear. In all other segments examined the absorption rate was much greater and curved lines were obtained. The half-lives of the initial log-linear decrease were from 15·14 to 28·69 min (Table 1). The disappearance of exaprolol from duodenal and jejuno-ileal segments was faster than from the caecum and colon.

Table 1. Parameters of exaprolol absorption in rats. Results are the means ± s.d. of 4 determinations.

	$k_{a(dis)}$ (min^{-1})	$t_{\frac{1}{2}(dis)}$ (min)
Stomach	0·0069 ± 0·00276	100·43 ± 40·17
Duodenum	0·0457 ± 0·00828	15·14 ± 2·739
Jejunum and ileum	0·0439 ± 0·00391	15·77 ± 1·404
Caecum	0·0241 ± 0·00299	28·69 ± 3·553
Colon	0·0370 ± 0·00184	18·71 ± 0·930

The changes in the haemodynamic characteristics of rats exposed to the stress of the absorption experiment are summarized in Table 2. It can be seen that combined effects of anaesthesia, opening of the abdominal cavity and intestinal manipulation induced a deterioration in all the haemodynamic parameters measured. Thus, the intestinal circulation cannot be considered to be constant in the course of the absorption experiment.

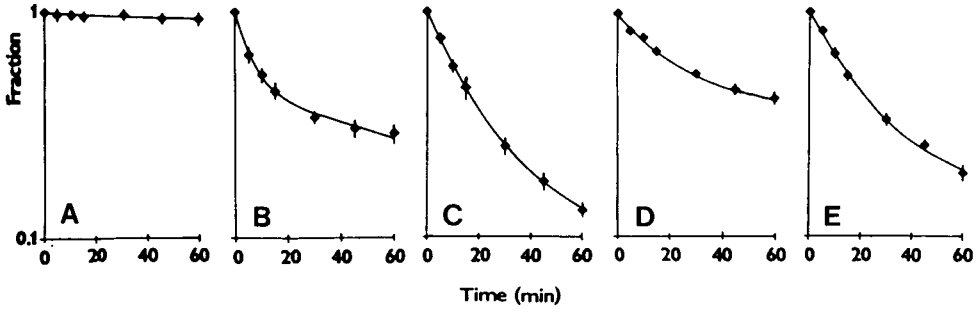

FIG. 1. The exaprolol amount–time curves for various segments of the gastrointestinal tract of rats (means ± s.e.m.). A stomach, B duodenum, C jejunum and ileum, D caecum, E colon.

Table 2. Haemodynamic parameters of experimental rats (n = No. of determinations; means ± s.e.m.).

	n	Start of absorption study	30 min after first injection
Cardiac output (ml min^{-1}/100 g)	6	31·00 ± 4·36	16·53 ± 1·55‡
Stomach (ml min^{-1} g^{-1})	5	0·576 ± 0·125	0·249 ± 0·015‡
Small intestine-upper third (ml min^{-1} g^{-1})	5	3·655 ± 0·575	1·788 ± 0·121‡
Small intestine-middle third (ml min^{-1} g^{-1})	6	1·396 ± 0·180	0·815 ± 0·102‡
Small intestine-lower third (ml min^{-1} g^{-1})	7	1·029 ± 0·116	0·628 ± 0·074‡
Caecum (ml min^{-1} g^{-1})	4	0·805 ± 0·017	0·315 ± 0·053‡
Large intestine (ml min^{-1} g^{-1})	6	0·432 ± 0·051	0·318 ± 0·035‡
Mean arterial blood pressure (mmHg)	7	107·4 ± 0·9	69·05 ± 1·86‡
Systolic blood pressure (mmHg)	7	138·3 ± 0·99	104·8 ± 2·87‡
Diastolic blood pressure (mmHg)	7	91·4 ± 1·3	51·4 ± 1·8‡

‡$P < 0.05$.

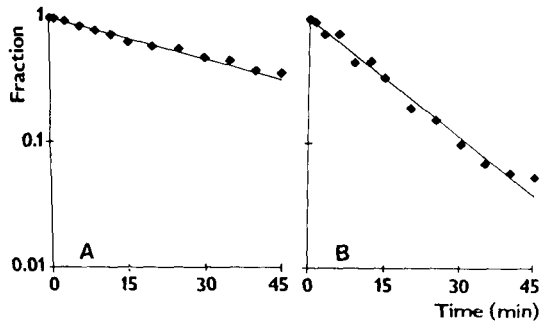

FIG. 2. Two examples of exaprolol amount–time curves for the ileum of the dog. A dose 0·33 mg ml^{-1} (Dog 1), B dose 0·033 mg ml^{-1} (Dog 4).

The disappearance plots of exaprolol from the ileum of dogs showed a monoexponential character (Fig. 2) for both doses used. The half-lives for the higher dose of exaprolol ranged between 8 and 27 min. Apparently, the rate of exaprolol disappearance (Table 3) was not related to its concentration in the loop. Within 45 min of the experiment the pH of the solution increased from 7·2 to 7·7. At this pH range, exaprolol, having a pK_a of 9·27, will be ionized from 90 to 99% ensuring good solubility.

Discussion

Although data concerning the absorption of exaprolol after oral administration to rats has been published (Trnovec et al 1982), detailed knowledge of the absorption topography was lacking. Exaprolol is a weak base (pK_a = 9·27 ± 0·06 at 25 °C) and its octanol–buffer distribution coefficient is 63 at pH 7·4. In spite of the nearly complete absorption, only 26% of the oral dose was systemically available because of extensive first-pass metabolism. Good absorption is one of the prerequisites of the enterohepatic circulation which has actually been established for exaprolol (Tomčíková et al 1984). It has been shown that exaprolol is also readily absorbed from the more distant parts of the gastrointestinal tract including ileum, caecum and colon. In agreement with the pH-partition principle, there was only a very small absorption from the stomach. Thus, exaprolol proved suitable for formulations with prolonged release.

Table 3. Parameters of exaprolol absorption in dogs. Results are the means ± s.d. of 3 determinations.

Dose (mg ml^{-1})	$K_{a(dis)}$ (min^{-1})	$t_{\frac{1}{2}(dis)}$ (min)
0·33	0·0482 ± 0·0283	17·883 ± 9·363
0·033	0·0630 ± 0·0122	11·310 ± 2·426

The in-situ method for assessment of drug absorption in rats is considered a good model that realistically reflects the conditions of absorption in-vivo (Doluisio et al 1969: Swintosky & Pogonowska-Wala 1982). The significant membrane storage of highly lipid soluble drugs during absorption (Doluisio et al 1970) that prevents a direct relation between the disappearance of the drug from the intestinal lumen and its absorption may be regarded as a disadvantage of this model.

It is suggested in this study that the deviation from linearity in the logarithm of the amount vs time plots in rats is probably due to reduced intestinal blood flow which occurred as a result of surgical stress. Exaprolol is a highly lipophilic drug and probably diffuses through the epithelium and interstitial space so rapidly that a nearly complete equilibrium between the blood and lumen is reached resulting in a blood flow-limited absorption process. Due to the inconsistency of the intestinal blood flow, the data obtained from the in-situ rat gut technique for rapidly diffusing lipophilic substances must be interpreted with care. In spite of the above limitations the $K_{a(dis)}$ values for the rat model derived from the initial portions of the curves compare very well with those from the dog experiments. This suggests that the same absorption mechanism occurs in both species. Absorption of propranolol in rats has been examined under similar conditions (Taylor & Grundy 1980), but the decrease in the amount of drug showed a monoexponential character, yet only four intervals were examined within 60 min in this study.

The absorption experiments with a chronic intestinal loop in dogs were carried out on conscious animals and reduction of the intestinal blood flow was avoided. The disappearance plots of exaprolol in dogs showed a monoexponential character. Good absorption of exaprolol from the dog ileum was recorded. The half-lives of absorption ranging between 8 and 27 min were comparable with those of propranolol (7·1 ± 2·0 min) (Taylor et al 1981) and no dose-dependence of exaprolol absorption was observed in the experiments reported.

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The effect of phenothiazine and dibenzazepine pretreatment on the metabolism of methamphetamine in rats

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There was marked variation between *p*-hydroxylation and *N*-demethylation of methamphetamine in rats. Within 24 h, 10·4, 24·7 and 4·1% of the administered methamphetamine was excreted in urine unchanged, *p*-hydroxymethamphetamine (*p*-OH-MP, free plus conjugated) and also as amphetamine, respectively. Treatment by imipramine, desipramine, chlorpromazine, perphenazine and propericiazine 8 h before the administration with methamphetamine completely inhibited the urinary excretion of *p*-OH-MP whereas the excretion of amphetamine was enhanced by about 700 to 800%. This effect was also observed in rats treated with imipramine 16 and 24 h before methamphetamine. Phenothiazine and dibenzazepine derivatives reverse the degree of *p*-hydroxylation and *N*-demethylation of methamphetamine in-vivo in rats.

In rats methamphetamine is first metabolized by *p*-hydroxylation and *N*-demethylation to give *p*-hydroxymethamphetamine (*p*-OH-MP), a major metabolite, and amphetamine, a minor metabolite (Caldwell et al 1972). Yamada et al (1984) have provided evidence for the involvement of cytochrome P-450 (P-450) and flavin-containing mono-oxygenase (FMO) in the rat liver microsomal *N*-demethylation of methylamphetamine. Yamamoto et al (1984) have shown that, in rats, microsomal *p*-hydroxylation of the drug is catalysed by different forms of P-450 and that the microsomal *N*-demethylation is mediated by both P-450- and FMO-dependent systems.

The present investigation sets out to show that whilst the phenothiazine and dibenzazepine derivatives inhibit *p*-hydroxylation (and certain forms of P-450) they do not inhibit *N*-demethylation.

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Materials and methods

Methamphetamine hydrochloride and amphetamine sulphate were gifts from Dainippon Pharmaceutical Co. Ltd and Takeda Chemical Industries, Ltd; imipramine hydrochloride, desipramine, chlorpromazine hydrochloride, perphenazine malate, propericiazine (10-(3-(4-hydroxy-1-piperidinyl)-propyl)phenothiazine-2-carbonitrile) were from Yoshitomi Pharmaceutical Co. Ltd and β-glucuronidase (H-1) was from Sigma Chemical Co. Ltd; *p*-OH-MP hydrochloride was prepared as described by Buzas & Dufour (1950).

Animal treatment. Male, Wistar albino rats (180–200 g) were housed individually in metabolism cages with free access to food and water. Groups of 6 rats were treated intraperitoneally (i.p.) 8 h before the i.p. administration of methamphetamine (10 mg kg^{-1}) with the following: 0·9% NaCl (saline) 0·2 ml, imipramine (40 mg kg^{-1}), desipramine (40 mg kg^{-1}), chlorpromazine (30 mg kg^{-1}), propericiazine (40 mg kg^{-1}), perphenazine (40 mg kg^{-1}). In one experiment, imipramine (40 mg kg^{-1}) was given 8, 16 and 24 h before methamphetamine (10 mg kg^{-1}). Urine samples were collected for 24 h. Urinary pH was recorded and the samples frozen at −15 °C until assayed.