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The absorption of β-adrenoceptor antagonists in rat in-situ small intestine; the effect of lipophilicity

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Intestinal absorption characteristics of eleven β-adrenoceptor antagonists were measured by monitoring their disappearance from in-situ intestinal loops in the anaesthetized rat. All have basic pK_a values of around 9·5 (with the exception of sotalol) but show a wide range of lipophilic character (octanol–water log P values from −0·79 to 3·65). The results show two types of absorption behaviour, indicating different mechanisms for 'hydrophilic' and 'lipophilic' β-adrenoceptor antagonists. The four most hydrophilic molecules (sotalol, atenolol, nadolol and practolol) show virtually identical absorption rate constants. Absorption is slow and relative rates in jejunum (mean pH 6·5) and ileum (mean pH 7·3) are not consistent with pH-partition (jejunum ≥ ileum). The more lipophilic members of the series (pindolol, timolol, metoprolol, oxprenolol, alprenolol and propranolol) are all absorbed much more rapidly. Absorption rate constant rises rapidly with log P and the expected pH effects are seen (ileum > jejunum). Acebutolol shows anomalously slow absorption for its log P value.

The β-adrenoceptor antagonists ('β-blockers') show a wide range of lipophilic character and have been classified in two groups as 'hydrophilic' and 'lipophilic' (Cruickshank 1980). All are absorbed from the intestinal tract in man and are active after oral administration, but there is evidence of marked quantitative differences in absorption properties. In general, absorption of the 'hydrophilic' compounds is slow and incomplete, and that of the 'lipophilic' compounds rapid and complete, but little detailed information on absorption characteristics is available. In this paper, we report absorption measurements in an in-situ anaesthetized rat gut preparation on eleven β-adrenoceptor antagonists which are, or have been, in clinical usage. Attempts are made to relate the measured absorption properties to octanol–water log P values and to what is known of their absorption in man.

Methods

(i) Physicochemical parameters

Octanol–water log P (log partition coefficient) values were obtained from the literature (Cruickshank 1980). Values of log D (log distribution coefficient) at the pH of the jejunum and ileum were calculated from log P, pH and pK_a.

(ii) Drug disappearance from intestinal lumen

The experimental technique was a modification of that reported by Dolusio et al (1969), using rate of drug disappearance from ligated in-situ segments of small intestine as a measure of absorption. Male albino rats (Alderley Park strain), 210–250 g, were fasted 18 h before use. Anaesthesia was induced and maintained with halothane (Fluothane, ICI). Following laparotomy, two 7·5 cm segments of small intestine were identified and loosely ligated, one in the jejunum approximately 2 cm distal to the ligament of Treitz and the other in the ileum approximately 2 cm proximal to the ileocaecal junction. Each segment was rinsed gently with isotonic sodium chloride (37 °C) to remove traces of food. Drug solution (0·5 mg ml^{-1} drug in 0·154 M sodium chloride), adjusted to pH 7 and warmed to 37 °C, was then introduced via a blunt needle, the ligatures being tightened to contain the drug solution. Drug remaining in the segment was recovered immediately after introduction (zero time) or at 20, 40 or 60 min after introduction, by excising the segment, with ligatures intact, cutting it open and washing out the contents with 0·154 M sodium chloride (1 ml). Recovered drug solution and washings were pooled and the pH measured. The solution was diluted to known volume and analysed for drug content by HPLC. A reverse phase column was used (5μ Hypersil C18, 0·5 × 10 cm), with a mobile phase of methanol–water–ammonia or methanol–water–trifluoracetic acid–sodium lauryl sulphate. An internal standard with a retention time similar to the drug was used in each case. Detection was by ultraviolet absorbance.

For most of the drugs, absorption measurements were made at zero and 20 min (rapidly absorbed molecules) or at zero and 60 min (slowly absorbed molecules), although time-course experiments were

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carried out in several cases to confirm that disappearance was by a first-order process. Absorption rate constants [k_a(dis)] were calculated from:

$$A_t = A_0 \cdot e^{-k_a(dis) \cdot t},$$

where A_0 and A_t are amounts recovered from the loop at zero and time t, respectively.

(iii) Drug appearance in systemic circulation

^{14}C-Radiolabelled atenolol (2-[4-(2-hydroxy-3-isopropylaminopropoxy)phenyl]-[2-^{14}C]acetamide) or propranolol (1-isopropylamino-3-[1-[1-^{14}C]naphthyloxy]propan-2-ol) were introduced into loops of jejunum or ileum (one loop only per rat) and samples of whole blood taken from the tail vein. A total radioactivity of 5 μCi per loop was used (drug concentration 0·5 mg ml^{-1}). Total radioactivity in the blood samples was measured by scintillation counting (Fisofluor 2, Fisons Ltd) following digestion in Soluene (Packard Ltd) and decolourization with benzoyl peroxide.

Results and discussion

Disappearance of drug from the intestinal lumen displayed first order kinetics, as illustrated in Fig. 1 for pindolol in the ileum.

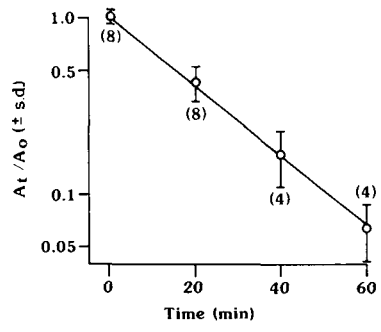

FIG. 1. Disappearance time course for pindolol in rat jejunum in-situ: plot of A_t/A_0 (log scale) versus time. Results shown are mean values ± standard deviation, with the number of determinations indicated for each time point.

Basic molecules such as these (pK_a = 9·5 except for sotalol) will be less ionized in the ileum (pH 7·3) than in the jejunum (pH 6·5) and should show faster absorption in the former. The measured values of k_a(dis), listed in Table 1, show that the picture is more complex than this and that there are marked differences between the 'hydrophilic' and 'lipophilic' β-adrenoceptor antagonists. Sotalol, atenolol, nadolol and practolol are all absorbed slowly and show virtually identical rate constants. Absorption is apparently slightly faster in the jejunum than in the ileum (ileum : jejunum ratio = 0·6–0·8) although this difference is not significant ($P > 0·05$). The 'lipophilic' group, with the exception of acebutolol, shows much faster absorption in both jejunum and ileum. Unlike the 'hydrophilic' group, ileal absorption is faster than jejunal absorption ($P < 0·05$), with an ileum : jejunum ratio of 1·5–2·5. Acebutolol is the only apparent exception to this pattern; it has a fairly high log P of 1·87, but shows slow absorption and an ileum : jejunum ratio of only 0·6. It clearly lies in the 'hydrophilic', rather than 'lipophilic' group, with respect to absorption characteristics. It is possible that this anomalous behaviour is related to the observation that acebutolol is actively transported from serosal to mucosal surfaces of the intestine (George & Gruchy 1979).

Disappearance models for intestinal absorption are now generally recognized to give an accurate indication of trans-membrane absorption of drug into the mesenteric and hepatic portal blood. Taylor et al (1981) proved this in the dog in-situ small intestine for atenolol, one of the drugs included in the present work. Rate constants for disappearance of drug from the intestinal lumen were found to be identical to absorption rate constants calculated from appearance in the systemic circulation. A similar attempt to prove the validity of disappearance measurements has been made in the current work for atenolol and propranolol, which represents the two different types of absorption behaviour seen. Blood concentrations measured after introduction of drug into jejunal or ileal loops give the profiles shown in Fig. 2. These confirm that ileal absorption is faster than jejunal absorption for propranolol, but that the rates are essentially equal for atenolol.

The relation between lipophilicity and absorption for this series of drugs is illustrated in Fig. 3; in this, log k_a(dis) is plotted against log D, which is log P corrected for ionization at the pH of the jejunum or ileum. The 'lipophilic' molecules show a roughly linear increase in log k_a with log D (for both jejunum and ileum) until log D = 0 where the slope begins to tail off, presumably due to a change from membrane control to aqueous diffusion layer control of absorption. This behaviour is similar to that seen by Higuchi et al (1979) for steroids in the rat small intestine and by Schoenwald & Huang (1983) for β-adrenoceptor antagonists in the rabbit eye. Extrapolation of this line to lower log D values would also fit the jejunal results for the 'hydrophilic' molecules. However, the ileal values do not fit and, overall, the results suggest that k_a(dis) is log D-independent for this group (including acebutolol), as indicated by the broken line in Fig. 3.

There is clearly not a simple relation encompassing both 'hydrophilic' and 'lipophilic' groups of β-adrenoceptor antagonists and the results are best interpreted in terms of separate absorption mechanisms for the two groups. The behaviour of the 'lipophilic' group is consistent with absorption by pH-partition. The ratio of ileal to jejunal k_a(dis) values (1·5–2·5) is not as high as the theoretical value of approximately 6 for bases with pk_a = 9·5, but this may be due either to lower intrinsic permeability in the ileum or to the fact that bulk luminal

Table 1. Absorption rate constants [k_a(dis)] for β-adrenoceptor antagonists in the rat in-situ jejunum and ileum

Drug	Log P	k_a(dis)[h^{-1}] Mean[1] ± s.e.m. jejunum	ileum	Ratio (i/j)	j/i[2] $P = 0.05$
Sotalol	−0·79	0·36 ± 0·05	0·26 ± 0·05	0·7	ns
Atenolol	0·23	0·35 ± 0·03	0·22 ± 0·05	0·6	ns
Nadolol	0·71	0·30 ± 0·06	0·17 ± 0·06	0·6	ns
Practolol	0·76	0·33 ± 0·05	0·26 ± 0·05	0·8	ns
Pindolol	1·75	0·99 ± 0·04	2·43 ± 0·09	2·5	s
Acebutolol	1·87	0·36 ± 0·04	0·23 ± 0·04	0·6	ns
Timolol	2·10	1·50 ± 0·06	3·21 ± 0·05	2·1	s
Metoprolol	2·15	1·41 ± 0·07	2·86 ± 0·09	2·0	s
Oxprenolol	2·18	1·73 ± 0·07	3·63 ± 0·09	2·1	s
Alprenolol	2·61	1·99 ± 0·08	3·94 ± 0·08	2·0	s
Propranolol	3·65	2·87 ± 0·06	4·11 ± 0·06	1·4	s

[1] n = 8–12.
[2] Significance of difference jejunum : ileum at $P = 0.05$, group t-test.

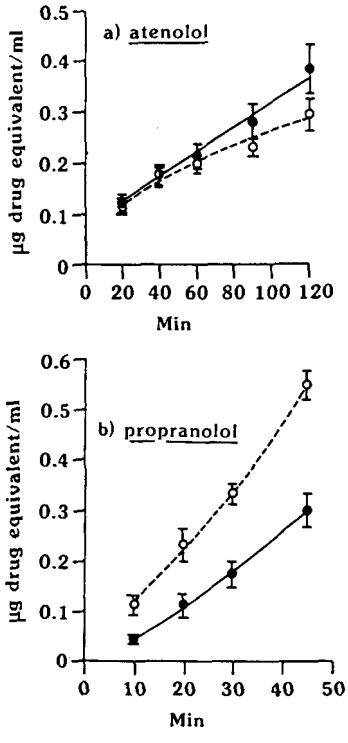

FIG. 2. Whole blood concentrations of radioactivity (expressed as µg equivalents ml^{-1}) after administration of [^{14}C]atenolol and [^{14}C]propranolol into in-situ loops of rat jejunum (—●—) and ileum (—○—). Results shown are mean values ± standard error; n = 6 for atenolol and n = 4 for propranolol.

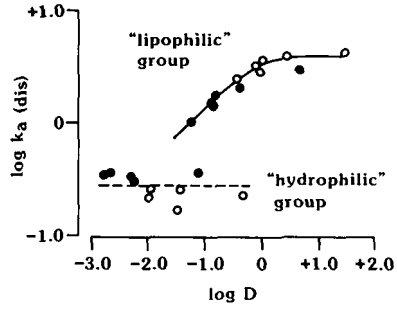

FIG. 3. Correlation between log k_a(dis) and log D for eleven β-adrenoceptor antagonists in the rat in-situ jejunum (—●—) and ileum (—○—).

pH values do not accurately reflect pH at the mucosal surface (Lucas 1983). The 'hydrophilic' molecules do not demonstrate pH-partition behaviour. Instead, they show the characteristics of aqueous phase transport (possibly via membrane pores), as proposed for β-lactam antibiotics by Ho et al (1983); absorption is independent of pH and log P, and the trend to reduced absorption in the ileum compared to jejunum reflects a general decrease in gut permeability in the distal direction. This route of absorption is highly dependent on molecular size and the similar k_a(dis) values measured for sotalol, atenolol, nadolol and practolol probably reflect their similar molecular weights. The transition between the two types of absorption behaviour (i.e. with increasing log D) is not clear from the present results and further work on adrenoceptor antagonists with log P values in the range 0·8–1·7 is required.

In man, the 'hydrophilic' β-adrenoceptor antagonists are characterized by incomplete oral absorption; overall absorption figures of approximately 60, 50 and 30% are recorded for sotalol, atenolol and nadolol (Dreyfuss et al 1977; McAinsh 1977; Fourtillan et al 1981), although practolol appears to be rather better absorbed (Reeves et al 1979). The 'lipophilic' molecules are all completely absorbed, with the exception of acebutolol (Collins & George 1976). This pattern is consistent with the results found in the present study in the rat in-situ intestine and

suggests that incomplete absorption in β-blockers is associated with the observed non-pH-partition route of absorption.

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Fat contents of meals and bioavailability of griseofulvin in man

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The bioavailability of griseofulvin was followed in twelve healthy volunteers by measuring the urinary excretion of the major metabolite 6-demethylgriseolfulvin, after each volunteer had ingested one 500 mg griseofulvin tablet under (1) fasting conditions, (2) immediately after a typical low-fat and (3) high-fat Nigerian meals. An increase of about 70 and 120% absorption occurred with the ingestion of the low-fat and high-fat meals respectively compared to the fasting state ($P < 0.01$). The maximum excretion rates of the free metabolite ($V_{max.}$) were also significantly increased ($P < 0.01$) following consumption of low and high fat meals. Our results thus suggest that the higher the fat content of the meals the higher the enhancement of the bioavailability of griseofulvin in man.

Griseofulvin is poorly soluble and a high fat diet has been listed among factors enhancing its absorption (Crounse 1963; Kabasakalian et al 1970; Khalafalla et al 1981). We have investigated the effect of Nigerian meals, composed of protein, carbohydrate and fat food sources on the bioavailability of griseofulvin in man as estimated from the cumulative urinary excretion of the major metabolite, 6-demethylgriseofulvin (6-DMG).

Materials and methods

The 6-DMG sample used for the preparation of calibration curve was isolated from the urine of a volunteer who had ingested one 500 mg griseofulvin tablet. The sample, purified and characterized had m.p. 286–288 °C (cf. 287 °C Arkley et al 1962). It gave a single spot (R_F 0·30) on a silica gel plate developed in chloroform–water–acetic acid (4 : 1 : 1) while griseofulvin had R_F of 0·90 on the same plate. The ^1H nmr of griseofulvin showed three methoxy groups at δ 3·63, 3·95 and 4·03 ppm ($CDCl_3$) whereas that of the 6-GMG sample showed only two methoxy groups at δ 3·65 and 3·83 ppm ($DMSOd_6$). The direct inlet mass spectrum of the sample gave M^+ at m/z 338 (36%) whereas that of griseofulvin gave M^+ at m/z 352 (42%). Sulphatase containing B-glucuronidase activity was obtained from Sigma Co., USA, 500 mg Grisovin tablets (Lot ILP 613) were kindly supplied by the Pharmacy Division of the University of Ife Health Centre.

The low-fat meal—corn gruel (pap) with fried bean balls (akara)—contained 29·3% fat calories while the high-fat meal, fried plantain with corned beef stew, contained 52·4% fat calories.

Twelve healthy male volunteers aged between 20–32 years (mean 24 ± 1) and 63 ± 2 kg (±s.e.m.) were instructed to abstain from taking any medicine or alcohol three days before the drug administration and throughout the trial. After fasting overnight each volunteer emptied his bladder just before taking the drug by mouth as one griseofulvin tablet (500 mg) with 200 ml of water. No liquid or food was allowed until 4 h after taking the tablet. The procedure was repeated a week later when the tablet was taken immediately after a low-fat meal, and after another week immediately after the high-fat meal. All volunteers received equal and identical meal portions. Total urine voided was collected at two-hourly intervals up to 12th hour and at the 24th hour after drug administration. The volume and pH of the urine samples were measured as soon as possible after collection. Samples were placed in well-stoppered tubes and stored at 5 °C until analysed for free 6-DMG according to Rowland & Riegelman (1973)

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