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EVIDENCE OF A SPECIALIZED TRANSPORT MECHANISM FOR THE INTESTINAL ABSORPTION OF BACLOFEN

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ABSTRACT

Absorption of the spasmolytic drug baclofen in three selected intestinal segments of living anaesthetized rats *in situ*, is shown to be a specialized transport mechanism obeying Michaelis-Menten kinetics. Equation parameters were calculated through different procedures, whose features are discussed. A computer method based on the integrated form of Michaelis-Menten equation which reproduces the entire time course of drug absorption from the data found in three intestinal perfusion series at different initial concentrations, yielded V_m and K_m values of 12·0 mg h^{-1} and 8·0 mg, respectively, in the mean segment of the small intestine, a rather selective absorption site for baclofen. Lesser but comparable absorption rates were found in the proximal and distal segments of the small intestine, whereas in colon, drug absorption was negligible. Baclofen transport was significantly reduced in the presence of the enzymatic inhibitor sodium azide. If these results were extrapolated to humans, they would explain the excellent bioavailability profiles reported for baclofen at normal doses in spite of its physicochemical properties, which do not favour passive diffusion. Based on the same principle, the administration of usual doses at shorter time intervals could be recommended, instead of high, when higher plasma levels at steady-state are needed. On the other hand, more than 8-h sustained-release preparations of baclofen should, probably, be avoided.

KEY WORDS Baclofen absorption Michaelis-Mentes kinetics Specialized absorption Non-linearity

INTRODUCTION

Baclofen (β-(p-chlorophenyl)γ-aminobutyric acid) is a widely used spasmolytic drug. It was developed in order to improve the inhibitory effect elicited by the structurally analogous physiological compound, γ-aminobutyric acid (GABA) on the nerve impulse transmission, in central nervous system.

Baclofen is reported to be rapidly absorbed and to have a very good extent of absorption when administered by the oral route in usual doses and conventional

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dosage forms.[1] When, however, higher doses of the drug must be given, the absorption period is considerably delayed and the extent of absorption is decreased;[2,3] this latter phenomenon has also been observed for experimental sustained-release forms of the drug. These observations, not yet satisfactorily explained, seem to indicate that absorption mechanisms of baclofen are complex and should be investigated further.

In order to gain insight into this behaviour, several series of experiences in rats were designed in our laboratory division, based on the perfusion of baclofen solutions at very different concentrations in selected rat intestinal segments. The results obtained were analysed in order to characterize:

1. the possible incidence of selectivity phenomena in baclofen absorption along the intestinal tract;
2. the existence of nonlinearities in absorption as a function of drug concentration in the perfusing fluids;
3. the features of the whole absorption kinetics of the drug as exactly as possible;
4. the effect of active transport inhibitors on the absorption of baclofen.

All these points can be of crucial importance since the chemical features of the drug (i.e. poor lipophilicity and amphoteric character) clearly do not favour its absorption in the intestine by passive diffusion processes governed by the common partition theory.

The use of the rat as experimentation animal is here justified on the basis of the parallelism observed in the absorption and disposition kinetics of baclofen in rats and humans.[1] On the other hand, rat preparations are perhaps unique to test the already outlined features.

MATERIALS AND METHODS

Absorption studies

The *in situ* rat gut preparation,[4] modified as previously reported,[5] was used for absorption tests, instead of the circulation methods, which have been widely used to characterize active absorption processes;[6,7] the reasons for the selection of this method will be justified later.

Wistar male rats weighing 200–300 g, fasted for 20 h, were anaesthetized 1 h before the experience by an intraperitoneal injection of ethyluretane (25 per cent, w/v). The whole length of the small intestine (about 1 m) was divided into three segments of equal lengths. Perfusion was performed in one of the segments or in the whole colon (5 rats each time); 5 ml of a buffered solution of baclofen (0·1, 0·5 or 2·5 mg ml^{-1} for small intestinal fractions, and 0·1 mg ml^{-1} for colon) was perfused at 37°. The pH was adjusted to the mean value found for each intestinal segment in preliminary 5-rat experiences:[8] 6·7 for proximal, 7·6 for mean, 8·2 for distal, and 7·5 for colon. The concentration of drug remaining in the perfused

solution was measured every 5 min for a total time of 30 min, through samples of 0·1 ml. In the proximal segment tests, the biliary duct was ligated.

Water reabsorption was evaluated separately in 3 animals for each intestinal segment and each drug concentration according to a previously reported procedure.[5] Baclofen amounts remaining in the intestinal lumen at each time unity were accordingly corrected. In order to prevent membrane adsorption effects,[4] only the samples obtained between 5 and 30 min were used for calculations; the initial nonperfused sample was excluded.

The same technique was used to evaluate inhibitory effects on baclofen absorption. Four separate series of tests, each in the mean intestinal segment (5 animals) were performed with solutions containing: (a) baclofen, 0·1 mg ml^{-1}; (b) baclofen, 0·1 mg ml^{-1} plus the inespecific active transport inhibitor sodium azide, 0·2 mg ml^{-1};[9] (c) antipyrine, 0·25 mg ml^{-1}; (d) antipyrine, 0·25 mg ml^{-1} plus sodium azide, 0·2 mg ml^{-1}. The two latter solutions were used as negative controls since antipyrine is known to be only passively absorbed.[7]

Analytical procedure

Intestinal baclofen samples were assayed for drug content by gas-chromatography according to the procedure described by Degen and Riess,[10] adapted as follows: a 0·1 ml volume of the sample was mixed with 0·1 ml of the internal standard solution (β-(2,4-dichlorophenyl)-γ-aminobutyric acid in 0·1N HCl, 100 ng per 0·1 ml) and 2 ml of methanol were added. The mixture was evaporated to dryness in a water bath, at 55°, under a stream of nitrogen. Then, 0·5 ml of the esterification reagent (a mixture of 1-butanol and acetyl chloride, 5/0·25, v/v) were added, and the solution was heated at 100° for 30 min, then cooled and evaporated to dryness under nitrogen at 55°. The sample should contain no more than 500 ng of baclofen; if the concentration is higher, it must be appropriately diluted. Five microlitres of pyridine, 1 ml of n-hexane and 10 μl of heptafluorobutyric anhydride were successively added to the residue, and the mixture was heated at 55° for 1 h in a water bath, then cooled, and 1 ml of 0·1 N NaOH added. The mixture was then vigorously shaken for 20 s and centrifuged for 5 min at 2000 rev min^{-1}. The organic phase (3 μl) was injected directly into the gas chromatograph. This procedure also worked well for baclofen samples containing sodium azide.

Antipyrine samples were analysed by liquid chromatography, according to the procedure recommended by Campbell,[11] with minor modifications. No attempts were made to quantitate sodium azide in the intestinal samples.

Fitting of models to data

Two types of fitting operations were developed. First, the remaining baclofen concentrations determined in perfusion fluids for each set of data (intestinal segments and initial baclofen concentrations, that is, ten data sets in all), were, as a preliminary step, fitted to linear kinetics (individual and average values). Secondly, on the basis of the three-concentration data sets found in each

intestinal segment (average values), *global fits* to Michaelis-Menten and first-order kinetics were achieved.

Preliminary fits. In order to detect possible selectivity phenomena in absorption and to assess nonlinearities, preliminary fits to the classical first-order equation were performed:

$$\ln A = -K_a \cdot t + \ln A_o \tag{1}$$

where A values are the remaining baclofen concentrations in the luminal contents at the sampling times, t, and A_o are the calculated intercept values at zero time. Absorption rate pseudoconstants, K_a, were then determined for each intestinal segment and each baclofen initial concentration data sets (mean of 5 animals) from the slope of the corresponding curves. The resulting K_a values were statistically compared in order to detect possible selectivity and nonlinearity phenomena in absorption. Similar comparisons were made between pseudoconstants found for baclofen and antipyrine data sets intended to look for absorption inhibition phenomena.

In addition, zero-order fits according to the equation:

$$A = -K_o \cdot t + A_o \tag{2}$$

were performed. These values will be necessary to develop further fitting operations, as will be pointed out later.

Global absorption kinetics. Fittings to Michaelis-Menten and then to global first-order kinetics were assayed according to several procedures and criteria:

1. *Direct differential method.* A direct fitting procedure was assayed based on the differential form of the Michaelis-Menten equation:

$$\frac{\Delta A}{\Delta t} = -\frac{V_m \cdot A_m}{K_m + A_m} \tag{3}$$

where ΔA is the decrease in baclofen concentration between the A value found at a given time and the following, for a given set of data (i.e. the difference, in $\mu g\,ml^{-1}$, between the concentration found at 20 min and that found at 25 min); Δt is the time that elapses between samplings (5 min in all cases), and A_m represents the concentration calculated at the mean time interval (i.e. 7·5 min for the values between 5 and 10 min) according to the best apparent kinetics previously assayed (first or zero order). The Multi program[12] was used for fitting equation (3) to these data (Simplex and Marquardt algorithm); calculations were performed globally for all remaining concentrations found in each particular segment by using the average values at each time unity (mean of 5 animals).

Based on the same principle but employing A_m values found by applying apparent first-order kinetics in the preliminary adjustments, global fits to the classical differential first-order expression:

$$\frac{\Delta A}{\Delta t} = - \overline{K_a} \, . \, A_m \tag{4}$$

were performed and compared with those found for equation (3). In equation (4), K_a represents the first-order absorption rate constant which globally satisfies the data found for all baclofen concentrations in the same intestinal segment.

2. *Linear differential transforms.* The classic Lineweaver-Burk procedure was also used to fit $\Delta A/\Delta t$ and A_m data sets calculated as above, through the equation:[13]

$$\frac{1}{\Delta A/\Delta t} = \frac{K_m}{V_m} \cdot \frac{1}{A_m} + \frac{1}{V_m} \tag{5}$$

The Eadie-Hofstee method[14] was also applied, using the expression:

$$\frac{\Delta A}{\Delta t} = - K_m \, \frac{\Delta A/\Delta t}{A_m} + V_m \tag{6}$$

3. *Integral method.* The remaining average concentrations of baclofen, A, found at each sampling time, t, at the three different initial drug concentrations in each particular intestinal segment, were *globally* fitted to the integrated form of the Michaelis-Menten equation:[15]

$$t = \frac{1}{V_m} (A_o - A + K_m \, . \, 1_n \, \frac{A_o}{A}) \tag{7}$$

through a nonlinear least-squares fitting procedure based on the Multi program.[12] Preliminary parameter estimates were obtained by using the Simplex algorithm, and, further, the Marquardt algorithm was employed to obtain the final parameter values (± SD). A subroutine was elaborated and incorporated into the program in order to calculate the theoretical remaining concentrations, A, for given t, V_m, K_m, and A_o values, according to the iteration method, until a difference less than 0·0001 $\mu g \, ml^{-1}$ between the calculated and actual A values was found.

In this way, V_m and K_m parameters wholly satisfying the above mentioned A values as well as the corrected A_o intercepts for each concentration set were finally obtained. When these parameters have been selected, the computer provides the best time values for a given concentration of baclofen in the

intestinal samples, including the A_o intercepts. Thus complete and continous plots of A versus t can be found.

Based on the above principles, global fits to first-order kinetics were also assayed through the use of the rearranged form of the classical equation:

$$t = \frac{\ln A_o - \ln A}{\bar{K}_a} \tag{8}$$

The resulting fits were compared with those found for equation (7), in order to select the best model for baclofen absorption kinetics.

Statistical methods

To test global differences in absorption, the first-order rate constants found in all conditions in the rat small intestine were subjected to a two-way (intestinal segments and initial perfusion concentrations) ANOVA test. In order to assess selectivity phenomena in absorption (i.e. the existence of an 'absorption window'), the constants found at the same initial concentration in different intestinal segments were compared through a Peritz-test.[16] The same method was used to detect nonlinearities in drug absorption, through the comparison of the first-order apparent K_a values found in a particular segment at the three different initial drug concentrations used. Nonlinearity was also tested by comparison of the total areas under the concentration–time curves, AUC, calculated according to the classical equation:[15]

$$\text{AUC} = \frac{A_o}{K_a} \tag{9}$$

through a one-way ANOVA test and a subsequent Peritz-test. Nonlinearity was assumed to exist when significant differences between the selected parameters (mean of 5 animals), at least for two of the concentrations tested in each data set, were found.

In order to judge how good the fits are (Michaelis-Menten and first-order) and to select the best one to describe baclofen absorption kinetics, the Akaike information criterion (AIC) was used, according to the expression:[17]

$$\text{AIC} = n.\ \ln (d^2_r) + 2p \tag{10}$$

in which, n represents the number of experimental data in each set, (d^2_r) is the sum of squares of deviations between experimental and theoretical points (i.e. weighted residuals), and P is the number of equation parameters. The smaller AIC value indicates the best fit.

Inhibitory effects in baclofen absorption resulting from the metabolic intoxication of possible carriers were characterized by statistical comparison (t –

Table 1. Percent average baclofen concentrations (± SD) relative to the initial perfusion concentration (A_i) remaining in the intestinal lumen at each sampling time. In the two last columns, the corresponding first-order rate pseudoconstants calculated from equation (1) and the ratios of normalized areas according to equation (9), respectively, are shown

Intestinal segments	A_i mg ml^{-1}	Per cent remaining at each time unity (min)						Absorption rate pseudoconstants K_a, h^{-1}	Ratio of normalized AUC values
		5	10	15	20	25	30		
Proximal	0·1	81·66 (±2·72)	73·89 (±3·96)	67·93 (±3·35)	62·01 (±4·04)	56·55 (±4·70)	52·38 (±4·04)	1·07 ± 0·14	1·00
	0·5	82·05 (±4·58)	76·25 (±2·50)	70·37 (±4·17)	66·12 (±3·71)	61·92 (±3·97)	57·26 (±3·86)	0·85 ± 0·07	1·23
	2·5	83·17 (±3·62)	79·09 (±2·84)	74·93 (±2·51)	70·38 (±2·61)	66·65 (±1·90)	61·87 (±3·15)	0·71 ± 0·13	1·54
Mean	0·1	80·87 (±1·65)	73·26 (±2·35)	64·28 (±1·45)	56·81 (±1·96)	50·70 (±3·14)	44·74 (±2·06)	1·44 ± 0·10	1·00
	0·5	83·89 (±4·03)	75·50 (±1·29)	69·03 (±1·41)	61·59 (±1·77)	55·88 (±2·71)	50·16 (±2·46)	1·23 ± 0·08	1·17
	2·5	80·20 (±4·46)	76·01 (±4·32)	70·21 (±4·23)	66·42 (±4·28)	63·21 (±4·11)	58·55 (±3·97)	0·75 ± 0·08	1·76
Distal	0·1	78·85 (±3·82)	72·18 (±4·14)	67·27 (±4·20)	62·35 (±5·13)	57·89 ±4·66)	53·15 (±4·96)	0·93 ± 0·12	1·00
	0·5	80·92 (±2·88)	74·98 (±3·30)	70·30 (±2·00)	65·46 (±3·73)	61·68 (±3·70)	57·47 (±3·82)	0·81 ± 0·10	1·18
	2·5	82·21 (±1·97)	78·63 (±2·56)	74·92 (±2·97)	71·49 (±3·15)	67·50 (±3·67)	63·32 (±3·42)	0·62 ± 0·09	1·54
Colon	0·1	89·45 (±3·80)	88·45 (±1·78)	87·27 (±1·23)	85·94 (±1·51)	86·32 (±1·87)	85·39 (±2·26)	0·11 ± 0·07	–

test) between the first-order rate pseudoconstants found in the presence and in the absence of the inhibitor (sodium azide).

RESULTS

The average concentrations of baclofen remaining in the intestinal lumen samples at each sampling time (A values, mean of 5 animals, expressed as the percentages of the initial perfused concentration) and corrected for water reabsorption, are given in Table 1. Since the colonic absorption of the drug has proved to be negligible, only the diluted solution of baclofen (0·1 mg ml^{-1}) was perfused in this segment. The apparent first-order absorption rate constants, K_a, calculated according equation (1), as well as the ratio of normalized areas, AUC, previously calculated according to equation (9), are also shown in Table 1.

Statistical significance of the differences between K_a values found in the different intestinal segments at the same drug concentration, as well as in the same intestinal segment, as a function of baclofen concentration, are shown in Tables 2 and 3, respectively. In Table 3, statistical significance of the differences between AUC values are also analysed.

In Table 4, the V_m and K_m parameter values found according to the four selected methods of calculation are given. Statistical figures are also indicated. The apparent global first-order rate constants found according to the differential direct method (equation (4)) and the integral fitting procedure (equation (8)) are given in Table 5. In Figures 1 to 3, graphs are plotted according to the Michaelis-Menten differential fittings methods. In Figure 4, the plot for the integrated Michaelis-Menten equation is shown. In Figures 5 and 6, the first-order global

Table 2. Statistical comparison between absorption first-order rate pseudoconstants found in different intestinal segments at the same drug concentration in the perfusion fluids, through the Peritz F test

Perfusion concentration (A_i, mg ml^{-1})	Intestinal segments compared	Significance (p value)
0·1	Mean–Distal	<0·0001
	Mean–Proximal	0·0021
	Proximal–Distal	0·1752 (NS)
0·5	Mean–Distal	<0·0001
	Mean–Proximal	0·0004
	Proximal–Distal	0·4468 (NS)
2·5	Mean–Distal	0·2388 (NS)
	Mean–Proximal	0·3268 (NS)
	Proximal–Distal	0·5209 (NS)

Table 3. Statistical comparison between absorption first-order rate pseudoconstants, K_a, and areas under the concentration–time curve, AUC, found at different initial baclofen concentrations in the same intestinal segments, through a Peritz F test. Nonlinearities are clearly evidenced in all fractions of the small intestine

Intestinal segments	Compared baclofen perfusion concentrations (A_i, mg ml^{-1})	Significance (p values)	
		K_a	AUC
Proximal	0·1–0·5	0·0498 (NS)	0·0439 (NS)
	0·5–2·5	0·1355 (NS)	0·0256
	0·1–2·5	0·0005	0·0013
Mean	0·1–0·5	0·0192	0·0103
	0·5–2·5	<0·0001	0·0005
	0·1–2·5	<0·0001	<0·0001
Distal	0·1–0·5	0·1779 (NS)	0·1826 (NS)
	0·5–2·5	0·0934 (NS)	0·0363 (NS)
	0·1–2·5	0·0014	0·0063

Table 4. V_m and K_m parameter values (± SD) found through the four selected calculation methods in the intestinal segments tested and statistical figures for each fit

Intestinal segments	Fitting equation	Parameter values		AIC values
		V_m (μg ml^{-1} min^{-1})	K_m (μg ml^{-1})	
Proximal	3*	65·73 ± 12·02	4016 ± 850	−18·65
	5	44·27 ± 26·33	2534 ± 1610	−38·47
	5*	66·70 ± 13·60	3880 ± 892	−17·82
	6	39·95 ± 9·52	2111 ± 654	+100·12
	7*	61·20 ± 1·25	3581 ± 23·5	−103·96
Mean	3*	39·04 ± 5·53	1603 ± 293	−17·10
	5	37·03 ± 13·62	1497 ± 590	−54·79
	5*	48·39 ± 7·22	1974 ± 375	−15·42
	6	35·64 ± 5·89	1367 ± 311	+95·64
	7*	39·92 ± 0·46	1602 ± 14·7	−119·67
Distal	3*	52·89 ± 7·79	3443 ± 581	−24·45
	5	37·43 ± 9·69	2330 ± 620	−46·02
	5*	56·27 ± 8·95	3573 ± 645	−23·13
	6	38·58 ± 7·20	2292 ± 539	+92·89
	7*	55·03 ± 0·74	3576 ± 14·4	−125·67

*Weighted values ($1/y^2$).

Table 5. First-order absorption rate constants, $\overline{K}_a$, globally satisfying the data found in each intestinal segment. Statistical figures for each fit are indicated for comparison with those reported in Table 4

Intestinal segments	Fitting equation	Global first-order rate constant ($\overline{K}_a$, h^{-1})	AIC values
Proximal	4*	0·809 ± 0·044	-5·95
	8*	0·873 ± 0·041	-77·63
Mean	4*	0·925 ± 0·085	+8·88
	8*	1·138 ± 0·078	-54·61
Distal	4*	0·733 ± 0·041	-4·57
	8*	0·787 ± 0·035	-83·11

*Weighted values ($1/y^2$).

plots according to the differential direct method and the integrated method, respectively, are represented. Only the fits found in the mean intestinal segment, taken to be representative of the drug behaviour, have been displayed.

In Table 6, the remaining baclofen concentrations, as well as those found for the control drug, antipyrine, in the absence and in the presence of the inhibitor

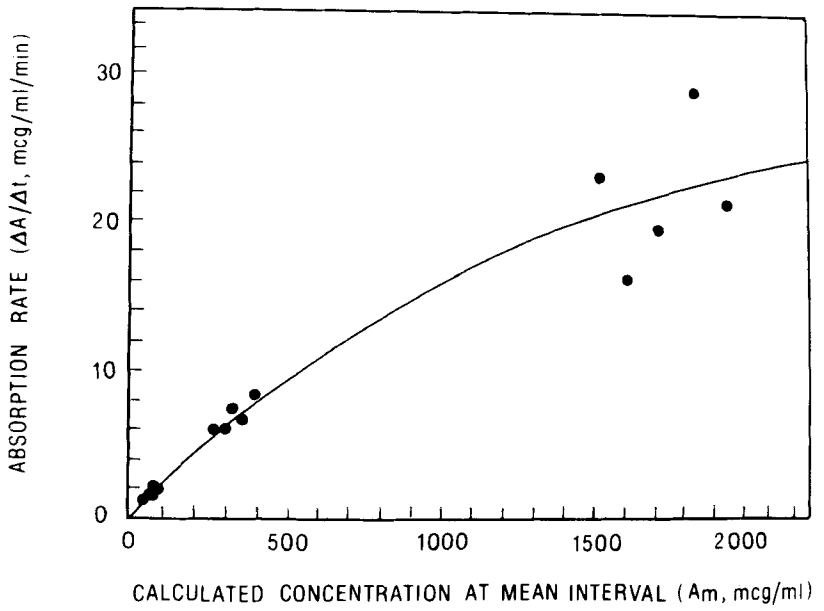

Figure 1. Plot of experimental data found in the mean intestinal segment according to the direct differential method (equation (3))

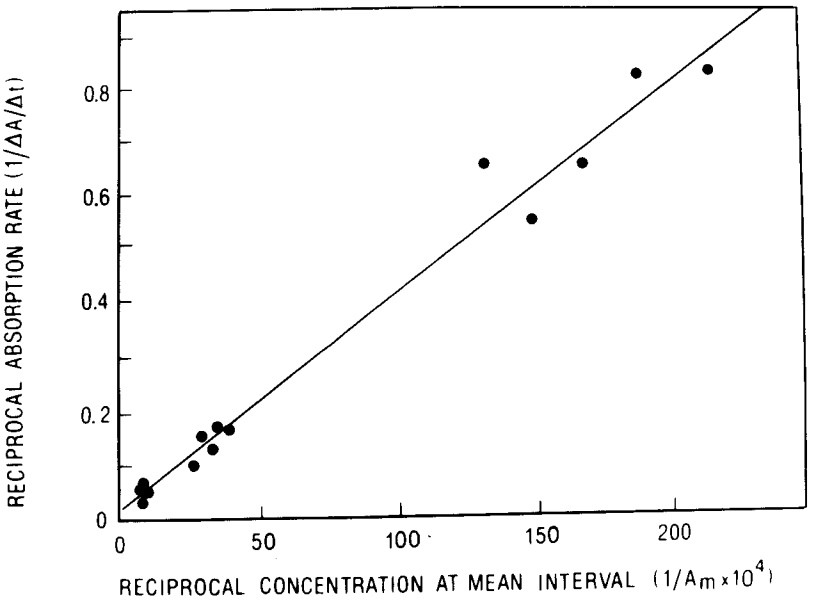

Figure 2. Plot found from the linear differential Lineweaver-Burk transforms (equation (5)) in the mean intestinal segment

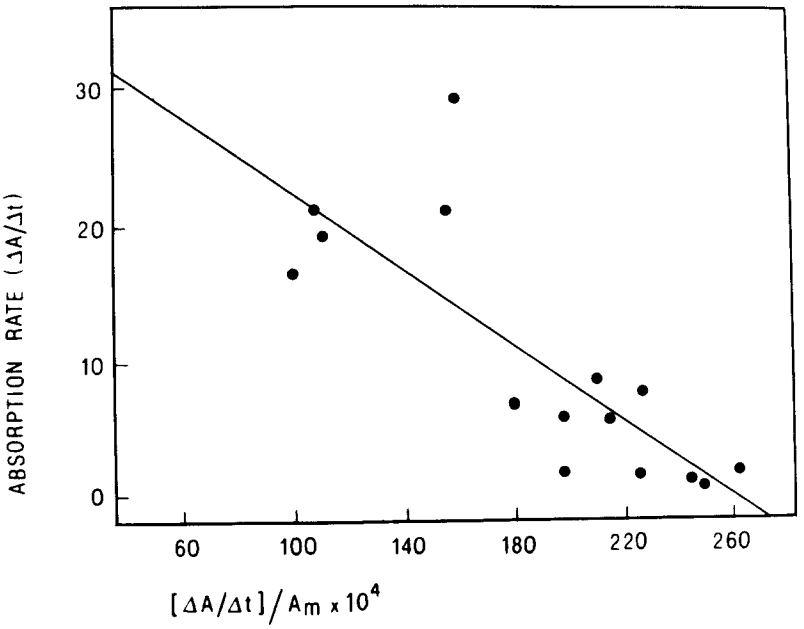

Figure 3. Eadie-Hofstee plot (equation (6)) found for the experimental data obtained in the mean intestinal segment

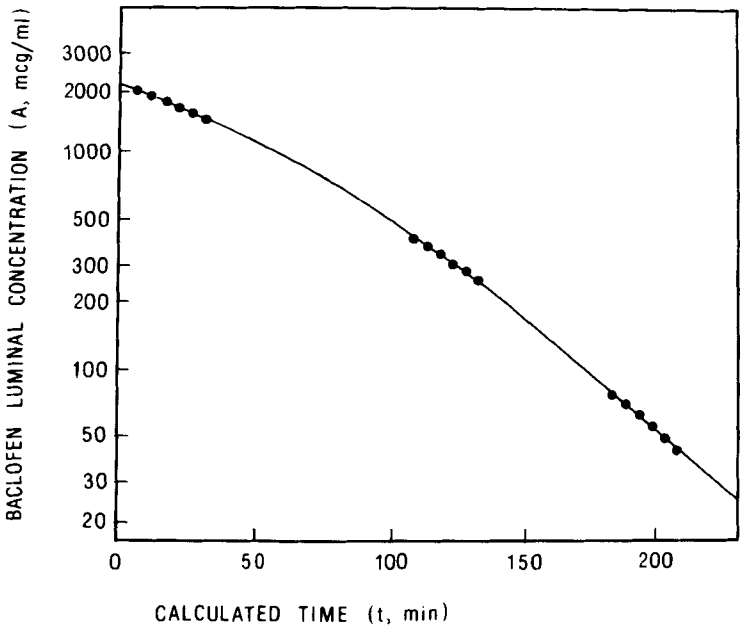

Figure 4. Semilogarithmic plot found from the integral Michaelis-Menten method (equation (7)) for the remaining concentration-time data in the mean intestinal segment. Note the excellent fitting characteristics

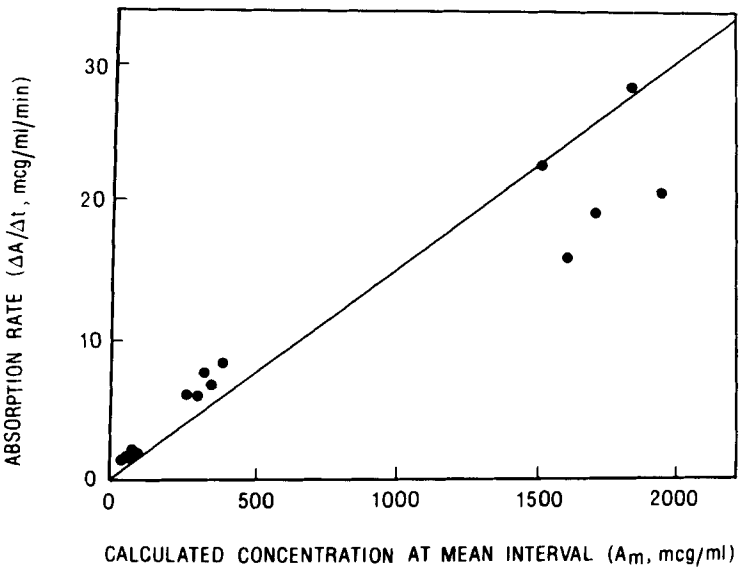

Figure 5. Linear plot found for global first-order kinetics (equation (4)) for the experimental data obtained in the mean intestinal segment. Compare the results with those in Figure 1

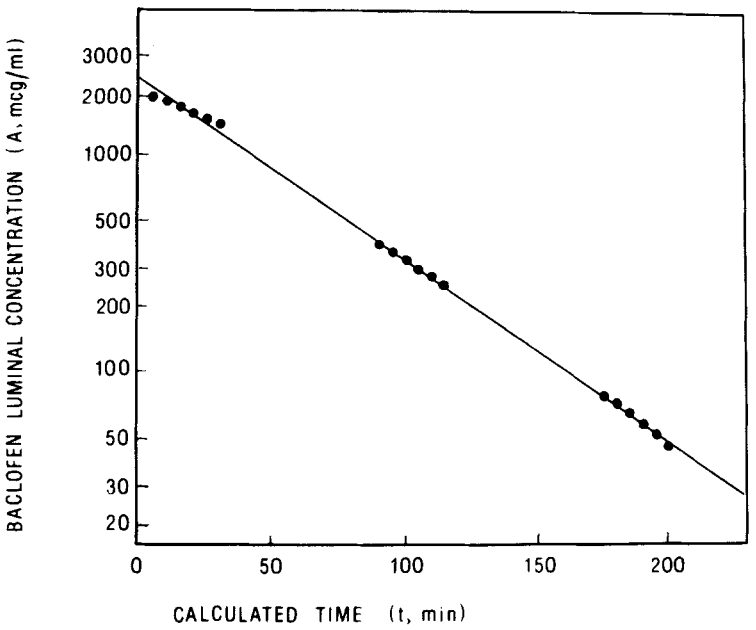

Figure 6. Semilogarithmic plot found from the global first-order equation (equation (8)) for the remaining concentration–time data obtained in the mean intestinal segment. Note that high concentration points are somewhat biased as compared with those shown in Figure 4

(sodium azide) are given, together with the first-order apparent rate constants found for each set of data. Statistical figures corresponding to these perfusion series are given in Table 7.

DISCUSSION

Experimental absorption technique

Intestinal single-pass or recirculation techniques[6,7] have been mainly used to characterize specialized absorption processes of drugs rather than *in situ* perfusion methods like that employed in the present investigation. It has been shown, however, that intrinsic permeability constants found by both types of methods, when normalized for perfused volumes and intestinal lengths, are virtually identical[7] provided that suitable corrections for water reabsorption are accomplished. Since the latter procedures will allow to absorption rates several-fold greater, nearer to *in vivo* values and much more suitable for kinetic calculations,[18] the *in situ* perfusion technique[4,5] was selected as routine working method, in order to characterize better the possible nonlinearities and to fit more easily absorption models to the experimental data.

Table 6. Percent average baclofen and antipyrine concentrations remaining in the intestinal lumen at the sampling times, relative to the initial perfusion concentration, with their standard deviations. Absorption rate apparent constants and correlation coefficients for each data set are also given

Sampling time (min)	Percentage of the drug remaining in the intestinal samples			
	Baclofen alone	Baclofen + azide	Antipyrine alone	Antipyrine + azide
5	81·20 ± 2·71	81·61 ± 1·15	74·25 ± 2·31	70·41 ± 1·47
10	72·07 ± 1·66	76·31 ± 2·22	58·92 ± 2·15	52·76 ± 1·89
15	63·52 ± 1·64	70·47 ± 1·43	46·99 ± 2·29	39·02 ± 2·38
20	56·44 ± 2·12	65·06 ± 1·87	37·22 ± 2·38	28·09 ± 2·40
25	50·51 ± 2·32	60·44 ± 1·86	29·73 ± 2·23	20·50 ± 2·05
30	44·51 ± 1·99	55·23 ± 2·39	23·71 ± 2·13	15·46 ± 1·91
$K_a(h^{-1})$	1·438 ± 0·14	0·937 ± 0·11	2·750 ± 0·20	3·697 ± 0·28
r	1·000	0·999	1·000	1·000

Selectivity phenomena in absorption

Through a two-way ANOVA test, significant differences were assessed between absorption rate pseudoconstants found in the three intestinal segments of the small intestine (F = 63·00). A subsequent Peritz F-test allowed to the comparison of the behaviour of a particular intestinal segment and that of any other. As shown in Table 2, no significant differences in K_a were found between proximal and distal segments of the small intestine at any tested concentration. In the mean intestinal segment, however, baclofen exhibits significantly higher K_a values than in either proximal and distal segments at the two lower concentrations tested. This may indicate that the mean intestinal fraction has a moderately higher absorption capacity for the drug. Table 4 shows that K_m figures found through the Michaelis-Menten equations are lower than those

Table 7. Statistical comparison between K_a values found in the presence and in the absence of sodium azide for test and control compounds. The influence of the inhibitor is clearly of opposite sign

Compared K_a values	Influence of azide on the K_a value	t value	Significance (p value)
Baclofen alone Baclofen + azide	Decreased	6·410	$< 0·01$
Antipyrine alone Antipyrine + azide	Increased	6·246	$< 0·01$

obtained in the two remaining intestinal fractions, a fact which tends to confirm this observation.

Nevertheless, it is our opinion that these differences are not strong enough to permit the mean intestinal segment to be designated as an 'absorption window'. It is possible that the rate and extent of absorption for baclofen are slightly higher in this segment, but if the above differences are compared with those found between all small intestinal segments and colon (Table 1), one can conclude that if there are absorption windows for the drug, they should, for practical purposes, to be identified as the whole small intestine.

Assessment of nonlinearities in absorption

Statistical analysis of the first-order rate pseudoconstants found in the preliminary fittings as a function of the concentration in the perfusion fluids in the same intestinal segment clearly shows that some nonlinearity exists. A two-way ANOVA test accounts for these differences (F = 39·38). A subsequent Peritz F-test shows that, as seen in Table 3, K_a values are significantly different for the extreme concentrations tested (0·1 and 2·5 mg ml^{-1}) in the proximal and distal segments, whereas in the mean fraction, the apparent absorption rate constants significantly differ for any tested concentration.

As far as the normalized areas are concerned, an one-way ANOVA test also shows existence of significant differences associated with the initial perfusion concentrations (A_i). Through a Peritz F-test, the statistical figures reported in Table 3 were obtained, which are similar in character to those found for rate constants.

The apparent K_a values tend to decrease as baclofen concentration in the perfusion fluid increases, contrarily to that which occurs with normalized AUC values. Therefore, the fitting to Michaelis-Menten equation is strongly recommended since this behaviour may indicate the existence of a specialized absorption mechanism.[15]

Evidence of a specialized transport mechanism

Through the observation of the statistical parameters found for Michaelis-Menten kinetics (Table 4) and first-order global kinetics (Table 5) for the two selected methods which can be compared (i.e. equation (3) with equation (4), and equation (7) with equation (8)), it becomes evident that the former is, by far, the best model for baclofen absorption. AIC values are clearly discriminatory. Even a visual inspection of some representative graphical plots reveals these features: whereas the fitting of equation (7) to experimental concentration/time data is completely reliable (Figure 4), it can be observed that first-order kinetics represented by equation (8) fits only the low-concentration/time data; the points corresponding to the higher drug concentration are considerably biased from the theoretical line (Figure 6).

Functionality of Michaelis-Menten fits

The heterogeneity of the variables used for fitting makes statistical comparison of the four selected methods unreliable in a strict sense; the statistical parameters reported in Table 4 are, therefore, indicative of the goodness of each fit but not of the relative goodness of the methods. In the opinion of the authors, however, the V_m and K_m values obtained with the aid of the integral method (equation (7)) are better for describing baclofen absorption kinetics since, for several reasons, the fitting to differential expressions (equations (3), (5), and (6)) implies modifying the true experimental data (i.e. A and t). This modification could be too drastic for the final parameters to be reliable. First, $\Delta A/\Delta t$ are incremental values, when the original equations assume that they are differential. Second, A_m values have only been approximated according to the more probable linear limiting kinetics (zero or first-order). Consequently, experimental errors and interindividual variations are probably potentiated by the differential calculation methods, whereas they could be minimized if the integral procedure, which works with the genuine variables A and t, is employed.

It could be pointed out that linear transforms of the differential Michaelis-Menten function (Equations (5) and (6)) without data weighing as currently used, give here parameter values at least questionable, perhaps only valid as initial estimates in most cases; in fact, the reliability of linear transforms of Michaelis-Menten equation has been criticized in specialized reports.[19,20] When equations (5) is used with suitable data weighing, according to the distribution of residuals ($1/y^2$), as has been done with nontransformed equations, most of the parameter values come closer to those obtained by the integral method, although, as can be expected, the statistical parameters become worse (Table 4). The direct differential method (equation (3)) provides here parameter values closer to those found from equation (7), but data scattering is still considerable, and could affect, in other cases and circumstances, the reliability of the final figures.

In view of these observations and considering some further features of the integral method (equation (7)), such as the excellent AIC parameters, the small standard deviation found for V_m and K_m values, and, from a practical viewpoint, the possibility of obtaining a global picture of the whole absorption process through the 'reconstruction' of the three partial perfusion tests (as shown in Figure 4), one can conclude that this procedure is perhaps of more general interest and usefulness than classical differential ones as a standard reference method to be applied to data sets that are similar in character to those found here. More will be said on this subject in forthcoming papers.

Taking as a reference the parameter values obtained by means of this method, it can be deduced from the similarity of the K_m values found in proximal and distal segments (Table 4) that an analogous and very general carrier system could be involved in baclofen absorption from these intestinal fractions (the small

differences could be related with pH). The K_m value found in the mean intestinal segment, which is considerably lower, could indicate that in this fraction a more specialized system (or systems) is also in operation, leading to an enhanced absorption of baclofen at low concentrations. Be that as it may, the above absorption mechanisms give rise to a lucrative drug absorption along the whole rat small intestine.

Absorption inhibition tests

The proceeding results clearly show that baclofen absorption works through a specialized, carrier-mediated mechanism. In order to gain an insight into the nature of this process, a series of perfusion experiences using sodium azide as the active transport inhibitor[9,21] were done in the mean intestinal segment. As a negative control drug, antipyrine, shown to be absorbed only by passive diffusion[7] was selected. As shown in Tables 6 and 7, baclofen absorption decreases significantly in the presence of the azide, whereas antipyrine absorption significantly increases. The reason for this latter effect, which has also been observed (although not to this statistically significant extent) with other inhibitors and xenobiotics,[22] is not known. Although further experiences are needed to clarify this effect, the results obtained here tend to indicate that an active transport mechanism could be involved in baclofen absorption. Tests are in progress to study competitive inhibition in baclofen absorption by using aminoacidic compounds resembling the drug in fundamental structure.

Practical implications

It is generally assumed that absorption processes in rat and man, in most instances, are basically similar.[23,24] In the case of baclofen, the similarities can be extended to the disposition of the drug.[1] It is, therefore, very likely that the kinetic features reported here have their counterpart in humans.

On this basis and in the opinion of the authors, practically all the observations in the literature concerning human baclofen intestinal absorption could be satisfactorily explained and interpreted in light of the reported results. The excellent biovailability profiles assessed for oral baclofen at usual doses in conventional dosage forms[1] should be interpreted to be no more than a consequence of the particular absorption mechanism shown here. The lipophilicity of the drug at the actual intestinal pH values is negligible;[8] therefore, it can be expected that passive permeation of baclofen across the intestinal lipoidal membrane will be virtually inoperative. On the other hand, the aqueous alternative route for passive intestinal absorption (i.e. aqueous pore diffusion) should be, for the most part, hindered by the molecular weight of the drug.[5,18] The negligible absorption rate constant found for baclofen in rat colon — where active absorption processes have never been identified — strongly supports this assumption. As a consequence, the good bioavailability reported in man should be entirely attributable to carrier-mediated absorption of baclofen, the only

mechanism which seems to be capable of allowing the drug to reach the splanchnic bloodstream.

One can also explain why baclofen doses that are considerable higher than normal are not suitable for obtaining higher steady-state plasma and greater therapeutic responses to drug.[2,3] The saturable character of baclofen absorption kinetics will allow the excess of drug to be emptied with the intestinal contents, and directly excreted in the faeces, with a reduction of the absorbed fraction of the dose (i.e. the extent of absorption). If higher steady-state plasma concentrations are needed, the administration of usual doses (up to 20 mg) at shorter time intervals could be recommended as a correct alternative clinical solution. This solution, in fact, has been suggested by some physicians[25] merely on the basis of clinical observations.

It should be pointed out, finally, that absorption in colon is crucial for reaching prolonged plateau levels of drugs with a half-life similar to that of baclofen.[26] Since colonic absorption is not, indeed, an operative process for this drug, it can be predicted that, if controlled-release formulations of baclofen are to be considered, they should be designed so that they would release most of the drug before reaching the ileal-caecal junction, i.e. within no than than 8 h.

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