

## TAURINE AND CAFFEINE-CONTAINING ENERGY DRINKS: LUMINAL PRESENCE OF TAURINE REDUCES BACLOFEN INTESTINAL ABSORPTION IN RATS

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### ABSTRACT

*The absorption of baclofen (BAC), a centrally acting antispastic agent with  $\gamma$ -amino acid structure as well as the influence of taurine (TAU) and caffeine (CAFF) on baclofen permeability was investigated by in-situ single-pass rat intestinal perfusion. Taurine is known to be transported by  $\beta$ -amino acid carrier, a transporter that is presumed to be shared by baclofen in its intestinal absorption. The aim of this study was to characterize the intestinal transport properties of baclofen in three different intestinal segments and to demonstrate the influence of co-administration of taurine as a competitive inhibitor of active baclofen absorption. Jejunum, ileum and colon of male White Wistar rats ( $n=4$ ) were perfused simultaneously. Baclofen concentration was 0.1 mM, a concentration, at which baclofen transport is not yet saturated. Following 30 minutes perfusion with pure baclofen in tyrode solution, 400mg/dl taurine (32 mmol/l), resp. 30 mg/dl caffeine (0.62 mmol/l) were added solely as well as in combination to the perfusion solution and the perfusion was continued in the same animal up to 90 minutes. Taurine and caffeine concentrations corresponded to those usually used in energy drinks. Additionally, baclofen permeability was directly studied using a typical energy drink (ED) Red Bull® instead of tyode. The perfusates were collected and baclofen concentrations were quantified by HPLC. The intestinal permeabilities ( $P_{eff}$ ) were determined on the basis of the differences between the inlet and outlet concentrations. Results from in-situ intestinal perfusions in jejunum showed that  $P_{eff}$  values*

*are approximately reduced to 19.5% following addition of taurine, 10.4% by addition of taurine plus caffeine. The energy drink reduced baclofen permeability to 13.9%. The effect of taurine and caffeine in ileum and colon was similar to those found in jejunum.*

## INTRODUCTION

During the last decade, numerous different taurine and caffeine containing beverages, so-called “energy drinks” appeared and were marketed in more than 50 countries worldwide, including Europe, the United States of America, and Australia. Nevertheless, the safety of these drinks, due to their high content of taurine and caffeine, is still under discussion. One of the components that do not belong to the group of ingredients regarded as nutrients is taurine (TAU), an endogenous sulfonic  $\beta$ -amino acid with highest concentrations found in retina (*Vinnakota et al., 1997; Militante and Lombardini, 1999*), brain and myocardium (*Huxtable et al., 1980*). Taurine has plenty of biological functions (*OFlaherty, 1997*).

Although there is a high need of taurine, the capacity of the human body to synthesize taurine appears low. Taurine is biosynthesized by two enzymes, the cysteine dioxygenase (CDO) and the cysteine sulfinic acid decarboxylase (CSAD) (*Bitoun and Tappaz, 2000*). The metabolic capacity of the rate-limiting enzyme in taurine biosynthesis, the cysteine sulfinic acid decarboxylase (CSAD) (*Wu et al., 1999*), indicates the low endogenous synthesis rate of taurine and illustrates the importance of taurine supplementation by nutrition.

Caffeine (CAFF), the second component of energy drinks, which does not belong to the group of nutritive constituents, is a naturally occurring alkaloid, is either consumed in food or beverages (coffee, tea, soft drinks) or as an oral medication, since it represents one of the most commonly used drugs in North America and other countries. The wide-spread occurrence of caffeine in plants and its use by a big part of the population reflects its relevance as a psychostimulant to increase reaction time and concentration ability. The metabolism of caffeine in the human body occurs

via different pathways and includes the involvement of various enzymes (*Rasmussen and Brosen, 1996*). Since taurine utilizes amino acid transporters in the intestine and since caffeine is extensively metabolized, these constituents might be a source of drug-“food” interactions and affect e.g. intestinal absorption of drugs with similar transporter and enzyme specificities.

A drug, the absorption of which might be affected by the amino acid-type compound taurine is baclofen. Baclofen [R/S-4-amino-3-(4-chlorophenyl) butyric acid, BAC], an analog of the inhibitory neurotransmitter  $\gamma$ -amino butyric acid (GABA), has been widely used in the oral treatment of spasticity resulting from multiple sclerosis or tetanus since 1967.

Regarding the intestinal absorption of baclofen and due to its amino acid structure interaction with various  $\alpha$ -,  $\beta$ - and  $\gamma$ -amino acids was studied, in order to elucidate possible transport systems. In *1995 Cercos-Fortea et al.* observed an incomplete inhibition of baclofen permeability upon addition of leucine, a substrate of the LNA-carrier (carrier for long neutral amino acids), due to a common carrier. *Cejudo-Ferragud, (1996)* focussed their study on the interaction of baclofen with phenylalanine. Different concentrations of phenylalanine were used (0 - 100 mM), nevertheless a complete competitive inhibition of baclofen absorption was not detected. *Moll-Navarro et al. (1996)* examined the influence of taurine, a drug known to be transported by an  $\beta$ -amino acid-specific carrier, on the insitu intestinal absorption of baclofen in rat perfused jejunum. Their study showed a saturable inhibition of baclofen permeation upon addition of different amounts of taurine concentrations, and they concluded that both compounds share one intestinal carrier. As the inhibition of baclofen absorption even upon high taurine concentrations was incomplete, an additional transport system for baclofen was presumed. Other studies concerning possible interactions with baclofen showed significant reduction of baclofen upon coperefusion with  $\beta$ -alanine (*Polache et al., 1991*)

and  $\gamma$ -amino butyric acid (GABA) (*Nacher et al., 1994*).

Based on the finding of *Moll-Navarro et al. (1996)*, indicating a possible interaction between baclofen and taurine, we hypothesized that interactions between baclofen and food or beverage constituents, respectively, might as well be possible. The aim of this study was to characterize the intestinal transport properties of baclofen in different intestinal segments by a rat intestinal perfusion study (*Barthe et al., 1999; Doluisio et al., 1969*) and to evaluate the inhibitory effect of taurine as a possible competitive inhibitor of active baclofen absorption and - in addition - to evaluate the influence of caffeine, the second component of energy drinks. In order to exclude effects of other constituents of energy drinks on intestinal baclofen permeability direct effect of Red Bull™ as a typical example, on baclofen permeability was examined in an additional step.

## MATERIALS AND METHODS

### Compounds and chemical reagents:

R/S-Baclofen was a gift from Ciba-Geigy (Basel, Switzerland). Taurine, caffeine and DL-chlorophenylalanine were purchased from Sigma Chemical Co. (St. Louis, USA). N-Acetyl-L-cysteine, o-phthalaldehyde and buffer components were obtained from E. Merck (Darmstadt, Germany). All other reagents were of analytical grade, and all solvents were of HPLC grade. Water was distilled prior to use.

### Equipment:

Intestinal perfusions were performed using an Ismatec MC-MSCA8/6 peristaltic pump (Ismatec, Glattbrugg-Zürich, Switzerland) with silicone tubings with outer and inner diameters of 4.0 and 2.0 mm, respectively.

### Perfusion procedure:

#### - Surgical procedure:

The surgical procedure was performed according to the method

published by *Doluisio et al. (1969)*. The rats (male White Wistar from Charles River German, (Sulzfeld, Germany), n = 4, body weights 350 - 400g) were fasted for 18 hours prior to surgery, but they had unlimited access to tap water. Following a brief inhalation of diethylether, anaesthesia was continued via intraperitoneal administration of ketamine (50 mg/kg BW) and Rompun<sup>TM</sup> (10 mg/kg BW). The anaesthetized rats were placed on a 37°C-heating pad to maintain body temperature. Intestinal segments of the rats were exposed by a midline intestinal incision, the selected gut segments were rinsed and cleaned with tyrode solution of 37°C to prevent extensive mucus secretion into the eluate during perfusion, and finally silicon tubes were attached to the three intestinal segments, which were selected for each of the rats (jejunum, 5-8cm, ileum, 3-7cm and colon, 1.5-2.5 cm).

- **Coperfusion with baclofen and potential inhibitors:- stepwise perfusion:**

The total perfusion time of 90 minutes was divided into 2 periods, each consisting of a 15 min period without monitoring to reach steady-state conditions and a 30 min period for baclofen  $P_{eff}$  determination in intervals of 5 min (*Hanafy et al., 2001*).

The procedure included the determination of baseline  $P_{eff}$  for a particular condition and  $P_{eff}$  upon coperfusion in three gut segments of one animal.

- **Perfusion solution:**

The perfusion solution consisted either of tyrode or an energy drink with 300 mg/l of caffeine and 4 g/l of taurine (e.g., Red Bull<sup>TM</sup>). In addition, the energy drink contained the following components: Glucose and saccharose (total carbohydrates 113 g/l), citric acid,  $\gamma$ -glucuronolactone (2.4 g/l), and various vitamins (niacine, vitamins B<sub>6</sub> and B<sub>12</sub>).

**A) Perfusions based on tyrode solution:**

Tyrode buffer consisted of NaCl (8.0 g/l), KCl (0.2 g/l), CaCl<sub>2</sub>  
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(0.2 g/l), MgCl<sub>2</sub> (0.2 g/l). NaH<sub>2</sub>PO<sub>4</sub> (0.04 g/l), NaHCO<sub>3</sub> (1.0 g/l) and glucose (1.0 g/l) adjusted to pH 7.0 with hydrochloric acid. The initial perfusate concentration of baclofen was 0.1 mM. Taurine and caffeine were added to the tyrode solution reaching the same concentrations as present in the energy drink, i.e. 4 g/l taurine (32 mmol/l). and 300 mg/l caffeine (0.62 mmol/l). The perfusion consisted of two perfusion periods, each containing different compounds, either alone (baclofen (BAC) or in combination (BAC/TAU, BAC/CAFF, BAC/TAU+CAFF). Furthermore, perfusions were performed (in 2 rats for each taurine concentration), where different taurine concentrations were investigated, in order to elucidate the saturability of the respective inhibitable process.

### **B) Perfusions based on a commercial energy drink:**

The energy drink was either used unchanged (pH 3.1) or adjusted to pH 7.0 with sodium hydroxide solution. The perfusion consisted of two perfusion periods with baclofen (0.1 mM) either in neutralized (pH 7.0) or non-neutralized energy drink (pH 3.07).

**Perfusion conditions:** The perfusate (drug-containing tyrode buffer or energy drink) was maintained at 37°C by keeping it in a water bath and delivered through the intestinal segments simultaneously at a constant flow rate of 0.2 ml/min. At the outlet, perfusate was quantitatively collected at intervals of 5 minutes and stored at -20°C until chromatography.

**Calculation of water transport and calculation of permeability coefficients:** Water transport was quantified by weight and volume measurements.

**Water fluxes were calculated from the following equation:**

$$\% \text{ Water transport} = 100 \frac{m_{in} - m_{out}}{m_{in}}$$

With  $m_{in}$  and  $m_{out}$  representing the weights of solution entering and exiting the intestinal segment. The correction of baclofen concentrations

for water fluxes was performed on the basis of the ratio  $m_{in}/m_{out}$ . Usually, water transport was in the range of 5 to 10%.

Intestinal permeabilities were calculated on the basis of the mixing tank model using the following equation that was initially described by *Sinko et al. (1991)*:

$$P_{eff} = \frac{v \cdot \left( \frac{C_{in} - C_{out}}{C_{out}} \right)}{2\pi r l}$$

Where  $v$  is the flow rate,  $C_{in}$  and  $C_{out}$  are the respective inlet and outlet concentrations of the drug containing perfusion solution, and  $r$  and  $l$  the radius and the length of the corresponding intestinal segment (average radius of the intestinal segments: jejunum, 0.21 cm, ileum, 0.21 cm, colon 0.23 cm).

### Bioanalytical assays:

**Baclofen in perfusate:** The intestinal perfusate samples were measured for baclofen content applying an HPLC method that included fluorescence measurement (*Herber, 2002*). To a 100  $\mu$ l aliquot of the perfusate samples, 100  $\mu$ l of internal standard (DL-chlorophenylalanine) and 800  $\mu$ l of water were added. To 50  $\mu$ l of this mixture 50  $\mu$ l of a methanolic sodium hydroxide solution as well as 250  $\mu$ l of water were added. This mixture was separated by HPLC on a Zorbax C8 column (250 \* 4 mm; 5  $\mu$ m, Bischoff, Leonberg, Germany) following a pre-column derivatization procedure using *o*-phthaldialdehyde and *N*-acetyl-L-cysteine at ambient temperature for 5 minutes yielding fluorescent isoindole derivatives (Figure 1). Elution was carried out using phosphate buffer pH 6.5 / methanol / THF (50:47.5:2.5, v/v/v) as mobile phase, which was pumped at a flow rate of 0.6 ml/min. The derivatization product was monitored using fluorescence detection with excitation at 345 nm and emission at 443 nm. A representative chromatogram is depicted in Figure 1. Average retention times were as follows:

baclofen, 10.6 min, DL-chlorophenylalanine, 17.57 min and 19.37 min. Variability of the assay was in the range of 2 - 10% for the whole linear range (5000 -50 ng/ml), the limit of determination was 20 ng/ml.

### Statistics:

For steady-state average  $P_{eff}$  values were calculated for each rat and each treatment period (data usually given as arithmetical mean  $\pm$  standard deviation ( $SD_{(n-1)}$ ). Mean values (and their standard deviations for different individuals were always calculated from these  $P_{eff}$  values.

Student's t-test (unpaired, two-tailed) was used for statistical analyses.  $P < 0.05$  was considered to be statistically significant (**Sachs, 1978**).

The trend-test of Cox and Stuart ( $p < 0.01$ ) was used to statistically prove an increasing baclofen permeability under the presence of the acidic native energy drink (pH 3.07) (**Sachs, 1978**).

## RESULTS

### Baclofen intestinal permeabilities:

Calculation of intestinal permeabilities ( $P_{eff}$  values) showed a high transport rate of baclofen in all intestinal segments (jejunum, ileum and colon). A similar transport rate of baclofen was measured for the two small-intestinal segments jejunum and ileum (jejunum:  $2.45(\pm 0.068) \cdot 10^{-4}$  cm/s; ileum  $2.47 (\pm 0.247) 10^{-4}$  cm/s), whereas baclofen permeability was found to be 50.8% higher in the colon ( $3.71 (\pm 0.017) \cdot 10^{-4}$  cm/s) (Fig. 2; Fig. 3 insert).

### In-situ perfusion study with taurine in tyrode solution (BAC/TAU):

In all of the three examined intestinal segments addition of taurine led to a significant decrease in effective permeabilities.  $P_{eff}$  values upon the presence of taurine decreased to 19.5 % in jejunum, 19.8 % in ileum, and down to 14.0 % in colon (Figs. 2, 3, Table 1). Baclofen permeability decreased to nearly the same value in all three gut segments upon coperefusion with taurine. i.e. the inhibition of baclofen permeability was strongest

in colon. On the basis of the Student's t-test ( $p < 0.05$ ; two-tailed, unpaired), the difference between the gut segments is no longer statistically significant in the group of rats investigated here ( $n = 4$ ). An explanation for this unexpected finding could be a higher density of carriers in colon.

The investigation of different taurine levels yielded a clear taurine-concentration dependence of baclofen  $P_{eff}$  (Fig. 4). It became obvious that the taurine concentrations of 40 mg/dl present in energy drinks and used in most of the current investigations lead to half-maximal transport inhibition in jejunum and ileum ( $IC_{50}$ ). The respective calculated  $IC_{50}$  for colon amounted 55 mg/dl.

#### **In-situ study with caffeine in tyrode solution (BAC/CAFF):**

Upon addition of caffeine (Fig. 2, Fig. 3, Table 1)  $P_{eff}$  values decreased to 45.3 % in jejunum and 46.6% in ileum and was hence in the same range for these two gut segments. Caffeine effect on baclofen absorption in colon was less distinct than in the two other examined intestinal segments (reduction to 65.0%).

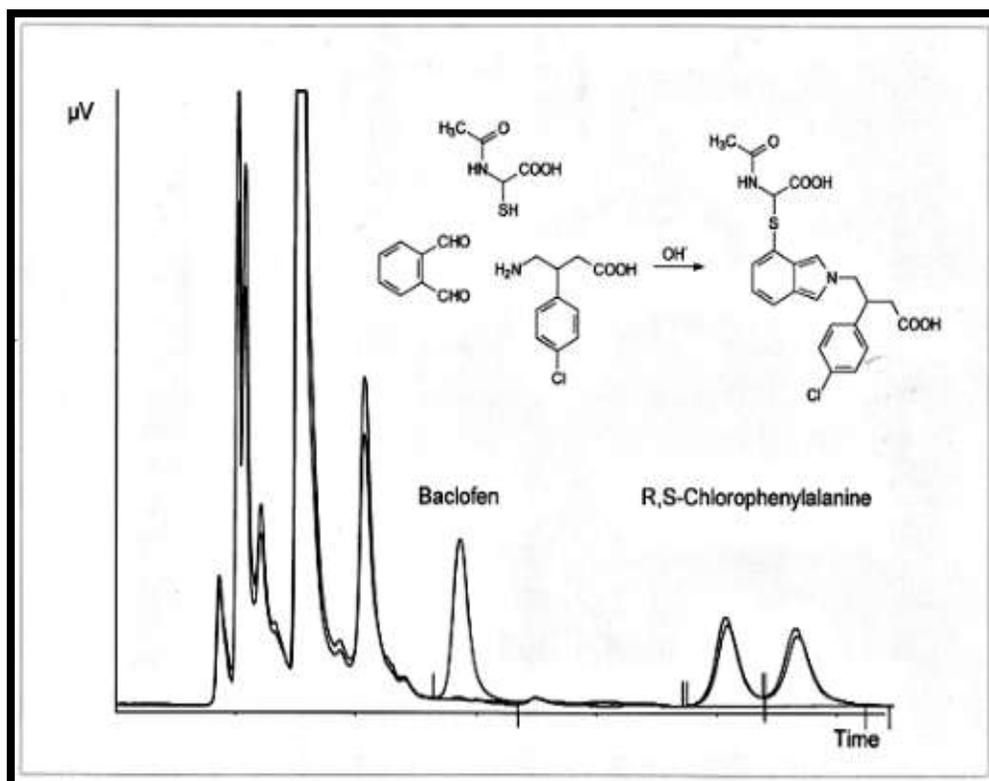
#### **In-situ study with taurine and caffeine in tyrode solution (BAC/TAU +CAFF):**

In agreement to the observations made with addition of the single ED components, also a mixture of taurine and caffeine containing tyrode led to a marked decrease of  $P_{eff}$  values, which exceeded the permeability reducing effect of the single components. In jejunum  $P_{eff}$  values decreased by 89.6 %, in ileum by 89.3 % and in colon by 92.9 %.

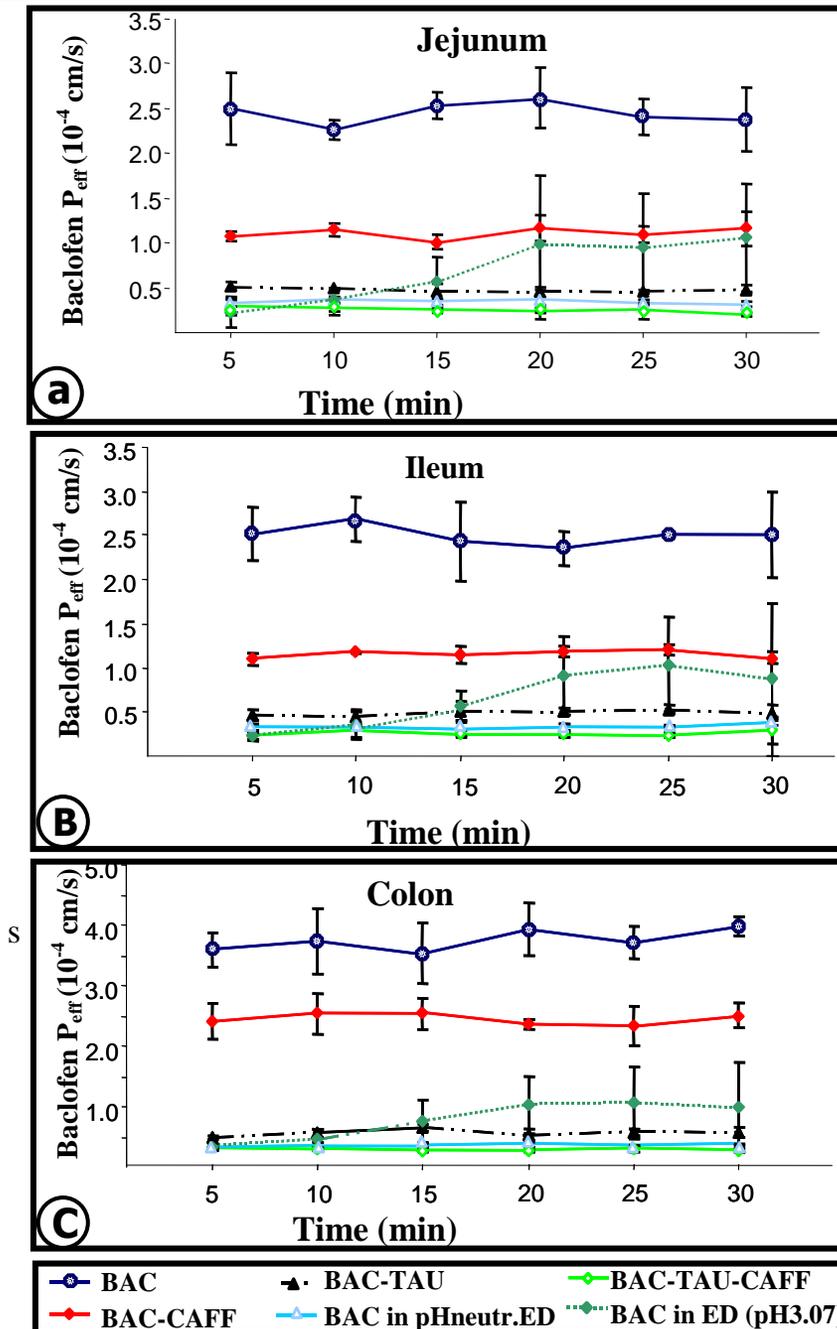
#### **In-situ study with energy drink (BAC/ED):**

The perfusion study carried out with baclofen dissolved in a taurine and caffeine containing energy drink (pH 7.0) showed similar results as those observed in tyrode solution, i.e., a marked and statistically significant decrease of baclofen permeability, when compared with the intestinal permeability of control baclofen. Baclofen permeability under the presence of the neutralized energy drink was slightly higher than in the tyrode buffer (difference statistically significant, t-test,  $p < 0.05$ ). When the study was

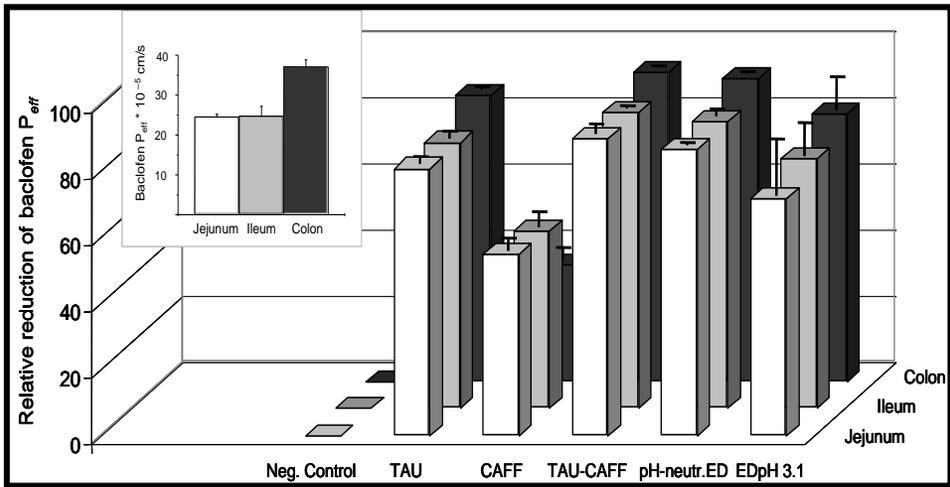
performed at two different pH values (at pH 7.0 with neutralized energy drink and at treatment at pH of 3.07), pH 3.07 baclofen permeability in 2 of the 4 animals was continuously increasing with time up to 20 or 25 minutes indicating irritations of the intestinal mucosa (Figures 2a, b, c). This effect was not detected at pH 7.0.



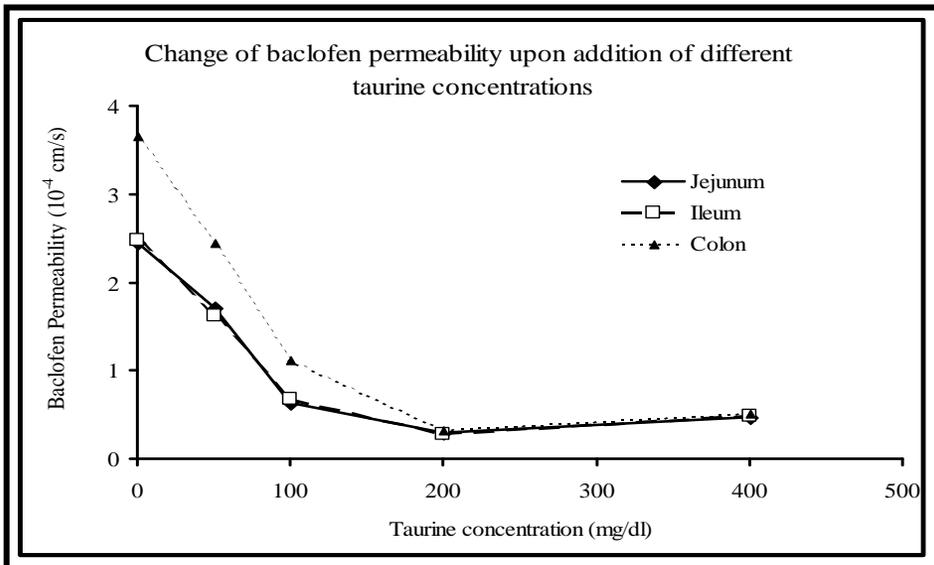
**Fig (1):** Representative chromatogram of baclofen and chlorophenylalanine as internal standard and a typical blank chromatogram as well as the derivatization scheme for baclofen with o-phthaldialdehyde and N-acetyl-L-cysteine yielding a fluorescent isoindole derivative (insert)



**Fig (2):** a-c Single effective intestinal permeabilities for baclofen as obtained during the steady-state period by the 5 min sampling interval in jejunum (a), ileum (b) and colon (c).



**Fig(3):** %-Inhibition of baclofen permeability by different taurine concentrations in three different gut segments (jejunum, ileum, and colon) as well as average baclofen intestinal permeabilities (P<sub>eff</sub>) in jejunum, ileum and colon (n = 4, values given as \* 10<sup>-4</sup> cm/s) (insert).



**Fig (4):** The extent of baclofen absorptive transport inhibition depends upon the luminal concentration of taurine.

## DISCUSSION

The in-situ intestinal perfusion represents a reliable method to study intestinal absorption of drugs and interactions with other compounds or food constituents in the different intestinal segments of the rat. Nevertheless, interpretation of intestinal absorption characteristics of baclofen as well as of taurine turned out to be difficult, as different transporters are believed to be responsible for drug permeation (*Nacher et al., 1994; Cercos-Fortea et al., 1995; Cejudo-Ferragud et al., 1996; Moll-Navarro et al., 1996*). So far, various transporters were studied for interference with the amino acid-type compounds baclofen and taurine, but it remains unclear, to which extent affinities to various carriers overlap. Moreover, as the endogenous synthesis of taurine is low and, therefore, the absorption of exogenous taurine is of great importance, the expression of the taurine transporter might vary depending on the dietary supply. *Shimizu and Satsu (2000)* reported of a down-regulation the intestinal taurine transporter (TAUT) if Caco-2 cells were grown in taurine-containing medium. They added taurine in concentrations of 1, 5, and 10mM to the medium and detected a concentration dependent decrease in taurine uptake. Regarding the growth medium containing 10mM taurine, relative taurine uptake was reduced by approximately 80%.

As described above, baclofen intestinal absorption is probably mediated by series of different amino acid transporters located in the intestinal membranes. In agreement with the known good bioavailability of baclofen, the observed intestinal permeabilities were fairly high.

At least one of the carriers involved in baclofen absorption from the intestine, the  $\beta$ -amino acid specific carrier, was inhibited by a high concentration of taurine as a competitive inhibitor in this study. The presence of taurine was consequently leading to a reduction of baclofen absorption

in all intestinal segments upon copercfusion with taurine. The strong inhibitory effect of caffeine on baclofen intestinal permeability was unexpected. One explanatory hypothesis is a mutual competition of baclofen and caffeine at the same intestinal carrier. As described by *Cejudo-Ferragud et al. (1996)* one possible way of baclofen intestinal absorption is its affinity to a transporter, which is shared by phenylalanine. *Kreydiyyeh reported in 1996* that caffeine-containing tea inhibited the mucosal uptake of phenylalanine, whereas the serosal transport remained unaffected. As the in-vitro activity of a  $\text{Na}^+/\text{K}^+$ -ATPase is even stimulated by caffeine, the authers assume, that the inhibitory effect of caffeine on phenylalanine absorption might be due to the elevated cAMP level by dissipating the sodium gradient needed for the uptake of phenylalanine. A comparabl mechanism might be possible for baclofen, as it shares the transporter with phenylalanine in its intestinal absorption. As alternative explanation a reduced blood flow as a consequence of the vasoconstrictory effect of caffeine is also possible.

When judging the importance of this food-drug interaction, it has to be taken into account, that taurine is a non-essential amino acid, which is synthesized in the body, mainly in the brain and in the liver. On the other hand, endogenous synthesis does not provide the human body with a sufficient amount of taurine. However, various nutrients, especially meat products, contain a considerable amount of taurine. The average uptake of taurine via nutrition has previously been estimated to amount to less than 200 mg per day (*Laidlaw et al., 1990*). The usual taurine content of a commercial energy drink is 1.0 g per can (400 mg/dl). This illustrates a yet unknown possibility of a “food”-drug interaction, if drug and drink are consumed in close proximity. Nevertheless, the taurine and caffeine effects are currently evaluated in rats in an in-vivo study to prove the clinical relevance of this food”-drug interaction. Furthermore, the influence of energy drinks on the  $P_{eff}$  values of various other drugs is currently screened. As result of the current study, it should be considered to add a

particular warning sign, in order to inform the consumers about the potential of "food"-drug interactions.

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