BUMETANIDE UPTAKE IN ISOLATED HEPATOCYTES OF THE LITTLE SKATE (RAJA ERINACEA)

M. Blumrich[1], E. Petzinger[1] and J. L. Boyer[2]
[1]Institut für Pharmakologie und Toxikologie, Justus-Liebig-Universität Giessen, 6300 Giessen, Germany
[2]Department of Medicine and Liver Center, Yale University School of Medicine, New Haven, CT 06510

In dog, rats and men the loop diuretic drug bumetanide is cleared from blood mainly through the liver (Busch et al., Arzneim. Forsch. Drug Res. 29: 315, 1979). Recent studies with isolated and cultured hepatocytes demonstrated sodium coupled carrier mediated bumetanide uptake via a high affinity and a low affinity transport system in isolated rat hepatocytes (Petzinger et al. Am. J. Physiol. 256: G 78, 1989; Föllmann et al. Am. J. Physiol. 258: C 700, 1990). Photoaffinity labeling of these putative transporter proteins identified a 52 - 54 KDa integral protein in rat liver basolateral plasma membranes (Petzinger et al. Eur. J. Pharmacol. Mol. Pharmacol. Sect. 208: 53, 1991). Other loop diuretics and bile acids competed with bumetanide for uptake and photoaffinity labeling suggesting a common uptake mechanism.

In isolated hepatocytes from the elasmobranch Raja erinacea only a single sodium-independent sinusoidal bile salt transport system has been characterized (Fricker et al., Am. J. Physiol. 253: G816, 1987; Smith et al., Am. J. Physiol. 252: G479, 1987). The elasmobranch organic anion transporter is thought to be an archaic transport system which may be present in mammalian species. It was therefore of interest to examine the mechanisms of hepatic uptake of bumetanide in liver cells from Raja erinacea.

Hepatocytes were isolated from male skates by a collagenase perfusion technique (Smith et al., J. Exp. Zool. 241: 291, 1987) and resuspended in elasmobranch Ringers. (^{3}H)-bumetanide uptake was measured by a rapid centrifugation method at 15 °C (Ballatori and Boyer, Am. J. Physiol. 254: R801, 1988). Uptake was linear for at least 60 s enabling initial uptake rates (V_i) to be calculated from this portion by linear regression.

Results indicate that bumetande uptake into isolated hepatocytes was saturable and energy dependent as well as temperature sensitive. However, in contrast to conjugated and unconjugated bile acids, bumetanide uptake was Na^+-dependent (see Table 1). When sodium ions were substituted in the incubation medium with choline, bumetanide uptake (V_i) was reduced to 58 %.

Uptake (V_i) into isolated skate hepatocytes at 15 °C

	TAUROCHOLATE (V_i ± SD)	CHOLATE (V_i ± SD)	BUNETANIDE (V_i ± SD)
$+Na^+$	20.9 ± 5.2	23.7 ± 5.4	23.7 ± 5.7
$-Na^+$	19.4 ± 4.7	22.6 ± 3.8	13.8 ± 3.8
Inhib.	7 %	5 %	42 %

Table 1. Initial uptake rates (V_i) of (^{3}H)-taurocholate, (^{14}C)-cholate and (^{3}H)-bumetanide in isolated skate hepatocytes 3 x 10^6hepatocytes/ml suspension were preincubated either in Na^+ or in choline elasmobranch Ringer solutions at 15 °C. V_i rates were determined by linear regression for the uptake of 11 nM (^{3}H)-taurocholate/10 µM taurocholate, 1.25 µM (^{14}C)-cholate/5 µM cholate or 60 nM (^{3}H)-bumetanide/7 µM bumetanide from the 15, 45, 75 and 105 seconds values. n = 6, X ± SD

Kinetic experiments (performed with hepatocytes pooled from livers of at least two skates) and resuspended either in Na^+ or in choline-elasmobranch Ringer media revealed the following kinetic constants:

- measured in Na^+-Ringer: K_m = 33 - 70 µM;
  V_{max} = 333 - 400 pmol x mg $^{-1}$ x min $^{-1}$

- measured in choline-Ringer: K_m = 33 - 66 µM;
  V_{max} = 250 - 250 pmol x mg $^{-1}$ x min $^{-1}$

- calculated Na^+-dependent portion: K_m = 52 - 79 µM;
  V_{max} = 100 - 200 pmol x mg $^{-1}$ x min $^{-1}$

As illustrated in Fig. 1 taurocholate competitively inhibited only the sodium-independent component of bumetanide transport, while the same bile acid was a non-competitive inhibitor in Na^+-Ringer solution. In turn bumetanide competitively inhibited (^{3}H)-taurocholate uptake (data not shown).

To further characterize the mechanism of bumetanide uptake, experiments were performed with 100 µM furosemide (a sulfamoyl benzoic acid derivative like bumetanide) and two bumetanide analogs PF-3034 (500 µM) and PF-2203 (500 µM). In rat liver cells these analogs almost exclusively inhibited cholate and bumetanide uptake but not sodium-dependent taurocholate uptake (Petzinger et al. Am. J. Physiol. 1992 submitted). In the present study with skate hepatocytes, these compounds inhibited taurocholate uptake but also preferentially the sodium-dependent bumetanide uptake system. Taurocholate (100 µM), cholate (100 µM) as well as probenecid (100 µM) preferentially inhibited sodium-independent bumetanide transport. BSP (25 µM), rose bengal (25 µM), bilirubin (25 µM), digitoxin (25 µM) and DIDS (100 µM) were strong inhibitors of both uptake mechanisms. In contrast aminoisobutyric acid (AIB, 2 mM) and para-aminohippuric acid (PAH, 1 mM) did not inhibit.

In summary, the results indicate that, unlike taurocholate transport in the little skate, a sodium-dependent as well as a sodium independent transporter is involved in bumetanide uptake into hepatocytes from Raja erinacea. The sodium-independent system is shared by bile acids, which is consistent with the hypothesis that this transport system is responsible for the uptake of a variety of different xenobiotics in lower vertebrates whereas the sodium-dependent bile acid transporter has evolved later in evolution. In contrast, the sodium-dependent transport system of the bumetanide uptake has evolved earlier. Therefore the liver cells of this marine species should be an excellent model to study phylogenetic aspects of bumetanide and organic anion transport.

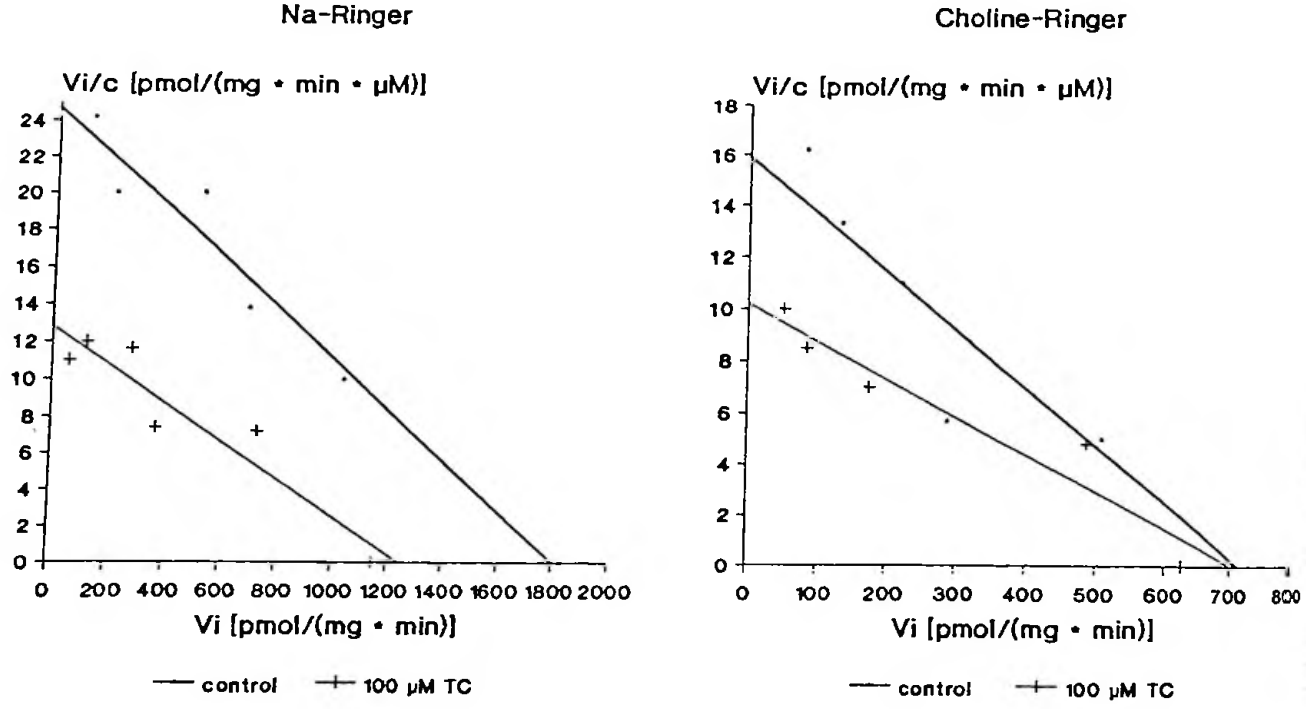

Figure 1. Eadie-Scatchard plot of the bumetanide uptake into freshly isolated skate hepatocytes in the presence of 100 µM taurocholate. In sodium elasmobranch Ringer solution (left figure) taurocholate non-competitively inhibited uptake while competitive inhibition was demonstrated in choline-Ringer (right figure). Each experiment was performed in duplicate with at least three different cell preparations.

Supported by Sonderforschungsbereich 249, project B3 of E. P., Giessen, Germany and DK-34989.