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Bile Synthesis Peculiarities Following Changes in the Functional State of the Endothelin Receptors

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Abstract

Endothelin-1(ET-1) regulates a variety of biochemical processes in liver. However, there is no clear view concerning endothelin receptors participation in the regulation of qualitative and quantitative characteristics of liver secretory function. The purpose of this study is to evaluate the choleretic effect of ET-1 and to determine the role of ET_A receptors functional state in mediating the effect of ET-1 on bile and its organic components secretion. Endothelin-1 and BQ-123 (cyclo-Asp-pro-Val-Leu-Trp) were intraportally injected. Bile flow, bile acid concentration and content, hydroxylation and conjugation coefficients were estimated. The results of this study showed that the secreted bile volume was decreased under the effect of endothelin-1and BQ-123, although this decrease was more prolonged and profound in BQ-123 treated animals. Concentration of taurocholates, glycocholic acid and free bile acids was increased in the endothelin treated rats. When BQ-123 was administered, an increase in glycochenodeoxycholic acid+glycodeoxycholic acid (GCDCA+GDCA) and the taurin-conjugated bile acids concentration was found, whereas the free bile acids concentration altered reversely. Coefficient of hydroxylation was diminished when entholelin receptors were blocked. Activation of endothelin receptors by exogenous endothelin-1 intensified bile acids biosynthesis via "neutral pathway,"involving microsomal oxidation enzymes. The present study concludes that the endothelin receptors blockade eliminated the regulatory function of endogenous endothelin and caused a shift in bile acids synthesis to mitochondrial enzymes through "acidic pathway".

Key words: Endothelin-1, BQ-123, Bile Acid, Coefficient.

1. Introduction

Regulatory peptide endothelin-1(ET-1), the predominant isoform of endothelin, is a potent vasoconstrictor agent that was originally isolated from bovine aortic and pulmonary endothelium. The translation of preproendothelin-1 mRNA results in the formation of a big ET-1 precursor, processing into ET-1(Yanagisawa *et al.*, 1998), activates G_i-protein–coupled 7-transmembrane domain receptors. There are different types of ET receptors that, with various levels of expression, are distributed in divers tissues in human and animal organs (Kawanabe and Nauli, 2011;Yanagisawa *et al*., 1998). ET_A receptor is selective for ET-1, whereas ET_B receptor reveals similar affinities with all isopeptides (Kawanabe and Nauli, 2011; Watts, 2010). Endothelin-1 has been recognized not only as a vasoconstrictor but also as a multifunctional agent. This peptide, through activation of ET_A receptors on intrahepatic vascular smooth muscle cells (Chan *et al*.,2004), the common bile duct (Huang, 2003), Kupffer cells (Yang *et al*., 2003) and hepatocytes (Hartman *et al*., 2010), elicits different pathophysiological effects. Moreover, a wide variety of biochemical processes in liver, including glycogenolysis, gluconeogenesis and hemodynamic action, are all regulated by endothelin (Monti *et al*.,2000).There are some investigations confirming an inhibitory effect of ET-1on bile secretion. According to Rodriguez *et al.* (2013), ET-1induces a dose-dependent decrease in bile flow in isolated perfused rat liver, while Tanaka *et al.* (1994), applying a similar experimental model, demonstrated that a low dose ET-1 increased bile acid-dependent bile secretion. However, these results do not give a clear view concerning endothelin receptors participation in regulation of qualitative and quantitative characteristics of liver secretory function.

The purpose of this study is to evaluate the choleretic effect of ET-1 and to determine the role of ET_A receptors functional state in mediating the influence of ET-1 on bile formation and biliary organic components secretion *in vivo* in rats.

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2. Materials and Methods

The present study has been conducted in acute experiments on 35 linear (male, 180-250g) Wistar rats, obtained from the Institute of Gerontology, Academy of Medical Sciences of Ukraine (Kyiv, Ukraine) after 18 hours of food deprivation. Rats were anaesthetized with ethyl urethane (100mg/100g rat body weight) which is sufficient for 3-4 hours of acute experiment. Common bile duct was then cannulated with polyethylene catheters to collect bile samples and register choleresis changes following laparotomy, respectively. Endothelin-1 (Sigma, USA, 0.1 μg/100g rat body weight) and ET_A-receptors antagonist, BQ-123 (cyclo-Asp-pro-Val-Leu-Trp; Sigma, USA, 6μg/100g rat body weight) were separately dissolved in 100 μl 0.9% sodium chloride and injected into the portal vein. According to previous studies, endothelin-1 in this concentration causes clear liver vascular movement that is an evidence for the active interaction between peptide and endothelin-1 receptors.

The animals of the control group were intraportally injected with 0.9% sodium chloride (100 μl/100g rat body weight). The secreted bile was collected every 30 minutes (consisted of three ten-minute fractions) during a 3-hour trial. The bile flow was measured by μl per g rat body weight.

Free and conjugated bile acids were divided and determined in each sample by a thin-layer chromatography method that was initially patented by Veselsky and colleagues and was completely described by Parchami Ghazaee *et al*. (2010). We divided the mixture of the bile acids into the following fractions: taurocholic acid (TCA), taurochenodeoxycholic acid+ taurodeoxycholic acid (TCDCA+TDCA), glycocholic acid (GCA), glycochenodeoxycholic acid+ glycodeoxycholic acid (GCDCA+GDCA), cholic acid (CA), chenodeoxycholic acid (CDCA) + deoxycholic acid (DCA). Bile acids concentration and content were estimated by mg% and μg/g rat body weight. Bile acid hydroxylation and conjugation coefficients were determined by calculating the ratios of 3-hydroxycholates to dihydroxycholates and conjugated to free bile acids contents.

All experiments were performed after entherahepatic circulation abortion, following stabilization of bile flow for 30 minutes at the beginning of experiment. The stability of rat body temperature was controlled by intrarectal thermometer. The statistical analysis was performed using the statistical package "Statistica."Data were expressed as means±SEM. Student's t-test for normally distributed values (Shapiro-Wilks W test) was used to compare variables between groups (p<0,05 was considered significant).

The study protocol was approved by the Institutional Review Board and Ethics Committee of Faculty of Biology, National Taras Shevchenko University of Kyiv.

3. Results

In the experiment on control animals, we observed a gradual reduction in secreted bile volume by 13.4% during the 3 hours of the experiment (from 0.298±0.09 μl/g in the first 10 minute interval to 0.258±0.11 μl/g in the last one). As illustrated in Figure 1, under the effect of endothelin, there was a considerable retardation in the level of bile secretion. A maximum choleresis reduction was registered in the 40th minute following the endothelin-1 injection by 15.6% (p<0.05) compared to control. Afterwards, a gradual bile flow restoration was observed and its level reached control values towards the end of the experiment. However, its level was lower than that in the first 10 minute interval by 8.6% (Figure 1).

Biochemical analysis of the half-hour bile samples evidenced that endothelin-1 exhibited a diverse effect on the concentration of different bile acids in rats. Although concentration of TCA was gradually reduced during the 3 hours of the experiment, both in control and ET-1 treated animals, its level in the sixth half-hour sample was higher by 9.3% (p<0.05) in the second group (141.5±2.3 mg% in control versus 154.7±2.8 mg% in ET-1 treated animals). However, in ET-1 treated total amount of TCDCA+TDCA was insignificantly higher in the majority of bile samples compared to control rats.

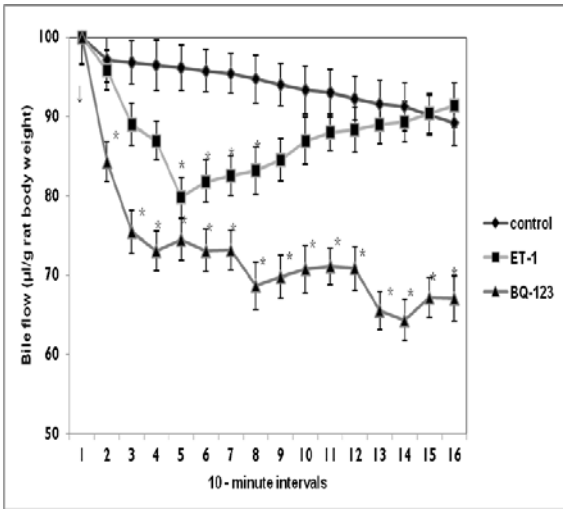

Figure 1. Effects of endothelin-1 and BQ-123 on choleresis, Mean±SEM; n=21, $^{*}p$<0.05 as compared to control rats.

The most drastic changes were observed in GCA concentration following ET-1 administration. Although the concentration of GCA in control animals was gradually reduced during the whole time of the experiment, it exceeded in the second half-hour, toward the end of the experiment when ET-1 was applied. Particularly, the GCA concentration increased by 12.3% (from 131.3±3.4 mg% to148.3±4.1 mg%; p<0.01), 19.7% (127.1±3.7 mg% to 152.1±4.3 mg%; p<0.01) and 16.3% (125.3±3.5 mg% to 145±7 mg%; p<0.01) in the fourth, fifth and sixth half-hour samples. At the same time, under the effect of ET-1,only a tendency for reduction in GCDCA+GDCA concentration was observed. Endothelin-1 caused an increase in CA concentration. Maximum changes were observed in the third and sixth half-hour bile samples by 20.6% and 19.8% (p>0.05), whereas, the CDCA+DCA concentration in ET-1 treated animals was next to control values (Table 1).

Table 1. Changes of the bile acids concentration (mg%) under the effect of endothelin-1 and BQ-123

Bile acids	Interval (min)	Bile acids concentration (mg%)		
		Control	Endothelin1	BQ-123
	30	168.6±5.5	173.1±4.7	167.8±6.2
	60	171.8±5.1	169.5± 3.9	182.5±5.3
TCA	90	163.1±5.2	155.1± 4.9	172.7±5.2
	120	159.0± 4.7	157.9± 4.2	177.6±5
	150	148.7±3.9	158.7± 4.4	174.3±4.9
	180	141.5±3.6	154.1±3.8*	177.3±4.7
	30	106.8±5.6	95.9± 8.9	103.7±5.5
	60	99.1± 6.1	104.6± 7.4	120.3±4.3
TCDCA+	90	95.7± 5.4	101.7± 5.2	112.3±4.7
TDCA	120	91.6± 5.2	96.8± 4.8	108.7±4.4
	150	86.7± 4.7	91.6± 4.3	109.4±4.2
	180	83.0± 4.3	92.1± 4.5	113.3±4.3
	30	135.8±5.4	132.7± 4.9	124.8±3.9
	60	138.0±5.8	136.5± 4.7	132.6±4.3
GCA	90	134.6±3.6	145.8± 3.8	132.9±4.2
	120	131.3±3.4	148.3±4.1*	134±3.7
	150	127.1±3.7	152.1±4.3**	129.9±4.1
	180	125.3±3.5	145.7± 3.9*	129.3±3.2
	30	36.8± 2.7	32.6± 2.9	31.7±2.1
	60	37.4± 2.5	28.1± 3.2	41.2±2.7
GCDCA+	90	35.3± 2.8	27.9± 3.0	43.3±2.6
GDCA	120	32.9± 2.6	26.8± 2.9	47.6±2.3
	150	29.0± 2.7	25.4± 2.7	46.7±2.7
	180	26.9± 2.5	24.3± 2.6	44.3±2.6
	30	23.2± 2.2	24.5± 2.3	22.7±2.4
	60	22.5± 2.1	25.4± 3.2	20.4±2.3
CA	90	21.8± 1.9	26.3± 2.9	21±2.4
	120	20.7± 1.8	23.2± 2.2	19.9±2.8
	150	19.1± 1.7	20.5± 1.9	17.8±2.5
	180	18.2± 1.9	21.8± 2.1	17.4±2.9
	30	7.4± 1.5	8.3± 1.7	6.9±1.3
	60	7.9± 1.8	8.1± 1.9	7±1.7
CDCA+	90	6.8± 1.2	7.2± 1.4	6.1±1.2
DCA	120	6.5± 0.9	7.4± 1.2	6.7±1.3
	150	6.3± 0.7	6.8± 1.1	5.7±1.1
	180	5.9± 0.8	6.3± 0.9	6.2±1.5

Mean±SEM; n=21, as compared with the changes in control rats. TCA, taurocholic acid; TCDCA+TDCA, mixture of tauro-chenodeoxycholicacid+ taurodeoxycholic acid; GCA, glycol-cholic acid; GCDCA+GDCA, mixture of glycochenodeoxycholic acid+glycodeoxycholic acid; CA, cholic acid, CDCA + DCA mixture of chenodeoxycholic acid +deoxycholic acid;* - $p< 0.05$; ** - $p< 0.01$

Peculiar changes in bile secretion were revealed when ET_A-receptors were blocked with ET-1 antagonist, BQ-123. It suppressed the action of both endogenous and exogenous endothelin-1 via this subtype of receptors. These functional changes in the ET_A-receptors state led to a more profound and prolonged decrease in choleresis. The bile secretion was decreased by 15.7% ($p<0.05$) immediately 10 minutes following the BQ-123 injection. Further, a choleresis reduction was observed in the next two ten-minute periods toward the end of the third period. It was decreased by 26.9% ($p<0.05$) compared to the initial state and 24.2% ($p<0.05$) compared to the control level. A maximum bile flow reduction was observed in minute 140 by 35.6% ($p<0.05$), compared to the initial state (Figure 1).

The chromatography analysis depicted considerable changes in the qualitative composition and the quantitative content of the bile acids in BQ-123 injected animals. The most marked increase in GCDCA+GDCA concentration was found in the fifth and sixth half-hour bile samples by 56% ($p<0.05$) and 60.6% ($p<0.05$) compared to the control values. At the same time, the concentration of GCA remained almost the same. The concentration of taurin-conjugated cholates was characterized by fluctuated changes during BQ-123 injection. Significant changes were only observed when it was enhanced; the TCA concentration in the fifth and the sixth half-hour samples was higher than in the control by 17.5% ($p<0.05$) and 25.3% ($p<0.05$). Also, the level of TCDCA+TDCA concentration was increased by 26.9% ($p<0.05$) and 35.5% ($p<0.05$). It is important to note that free bile acids concentration, simultaneously, conversely altered (Table 1).Figure 2 reveals the total contents of the different bile acids in the whole time of the experiment following ET-1 and BQ-123 injection.

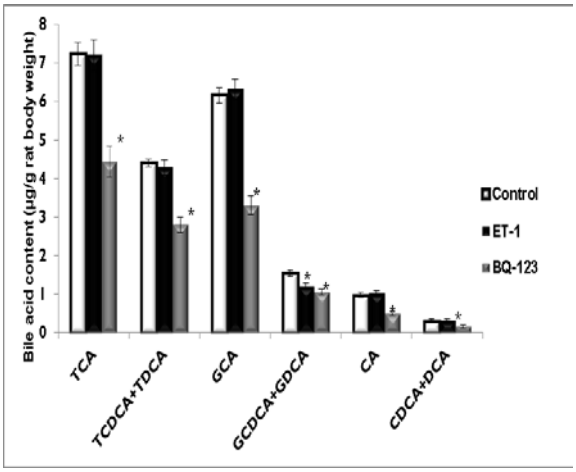

Figure 2. Bile acidscontent in endothelin-1 and BQ-123 treated rats; Mean±SEM; n=21 ;*$p<0.05$ compared to control

The dihydroxycholic acids concentration, in most bile samples, was significantly higher in BQ-123 treated animals compared to controls. This effect is clearly revealed in hydroxylation coefficient when the endothelin receptors were blocked (Table 2).

Table 2. Effect of endothelin-1 and BQ-123 on hydroxylation and conjugation coefficients of bile acids in rats

interval (min)		3-hydroxycholates mg%	Dihydroxy Cholates mg%	Hydroxylation coefficient	Conjugated bile acids mg%	Free Bile acids mg%	Conjugation ccoefficient
30	Control	327.6±3.8	151±2.7	2.2	443.1± 5.6	30.6± 2.7	14.5
	ET-1	330.5± 4.7	136.9±2.9	2.4	434.3± 6.5	32.8 ±2.9	13.2
	BQ-123	315.3± 4.8	142.3 ±3	2.2	428± 5.3	30.1± 2.8	14.2
60	Control	330.1±4.1	144.2±2.4	2.3	447.2± 4.7	30.4± 2.5	14.7
	ET-1	331.4 ±5.2	140.7±3.2	2.4	433.8± 6.3	33.5± 3.1	12.9
	BQ-123	335.7± 5.1	168.8±3.3	1.9	476.9± 4.7	27.6± 3.1	17.3
90	Control	319.5±3.6	137.8±2.2	2.3	428.7± 5.2	29.6± 2.1	14.5
	ET-1	327.2± 4.8	136.9±2.7	2.3	430.5± 5.9	33.3± 2.7	12.4
	BQ-123	326.8 ±4.4	161.8±2.7	2	461.3± 5.5	27.2± 2.4	17
120	Control	311.6±3.4	131.2±2.3	2.4	415.6± 4.8	27.2 ±2.2	15.3
	ET-1	329.4± 3.6	131.3±2.5	2.5	429.8± 5.4	30.6± 2.5	14.1
	BQ-123	331.8± 3.8	163.3±2.6	2	468.3± 5.2	26.8± 2.7	17.5
150	Control	294.9±3.2	122±1.8	2.4	391.5± 4.5	25.4± 1.9	15.4
	ET-1	331.3± 3.4	123.3±2.2	2.6	427.8± 4.7	27.2± 2.3	15.7
	BQ-123	332± 3.7*	161.8±2.5	2	460.3± 4.2	23.5± 2.5	19.6
180	Control	285±2.9	115.3±1.9	2.5	375.9± 3.8	24.1± 2.1	15.6
	ET-1	321.6± 3.5	122.4±2.3	2.6	416.5± 4.3	28.1± 1.8	14.8
	BQ-123	324.2± 3.7*	163.9±2.4	1.9	464.3± 4.4	23.8 ±1.9	19.5

ET-1: endothelin-1. Means±SEM; n=21, * $p<0.05$

4. Discussion

It is of great importance to clarify the bile synthesis peculiarities via evaluating the particular poly enzymatic systems efficiency in liver, which provides bile acid hydroxylation and amino acid conjugation processes, altering the bile colloidal system properties. Determination of hydroxylation and conjugation coefficients, as well as separate bile acid content under the effect of endothelin-1 and BQ-123, indicated significant disturbances in bile formation process.

The results revealed that endothelin-1 intensified biosynthesis of both free and glycine conjugated 3-hydroxycholates, synthesis of which is closely associated with the microsomal oxidation enzymes activity (neutral pathway), and so depends on tissue supply with oxygen. This agrees with the thesis that the oxygen consumption by the liver tissue is reduced after endothelin-1 administration (Baveja *et al*., 2002, Vlahcevic *et al*.,1997, Yanchuk *et al*.,2008).

Following BQ-123 injection, the coefficient of bile acids hydroxylation reduced from 2.2 in the first sample to 1.9in the last one, whereas in the control group, it increased from 2.2 to 2.5. This is evidences for the intensification of dihydroxycholates biosynthesis in ET_A-receptors blockade that is confirmed by a significant improvement in their glycine and taurine conjugated concentration. It is known that the biosynthesis of the initial chenodeoxycholic acid in liver is realized by an active participation of the mitochondrial enzymes that in our investigation is supported by a significant increase in its conjugates under the effect of BQ-123. Therefore, ET_A-receptors blockade points out an important role of endogenous endothelin-1 in neurohumoral regulation of bile organic components biosynthesis.

The study on rats has demonstrated that endothelin-1 and BQ-123 actively influence bile acids biosynthesis efficiency according to these metabolites content in bile. Endothelin-1 in applied dose promoted both biosynthesis of glycocholic acid and content of majority of bile acids in the whole time of the experiment, whereas endothelin receptors antagonist caused a reversed effect.

It is important to note that the significant and long-lasting choleresis retardation under the influence of BQ-123 is due to the partial removal of the bile acids from osmotic and diffusion processes in bile formation following a considerable decrease in their biosynthesis in hepatocytes.

We conclude that endothelin receptors activation by exogenous endothelin-1 provokes the short-term choleresis retardation which is accompanied by an intensification of bile acids biosynthesis via “neutral pathway, ”involving microsomal oxidation enzymes, the latter is proved by the alteration of 3-hydroxycholates to dihydroxycholates ratio and the increase of glycocholic acid content. Endothelin receptors blockade with BQ-123, which eliminates the regulatory function of endogenous

endothelin, causes a sharp and long-lasting decrease in bile flow, simultaneously, shifting process of bile acids synthesis, mainly to "acidic pathway," involving mitochondrial enzymes that is confirmed by enhancing the concentration of both free and conjugated dihydroxycholates.

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