Biochemical Pharmacology, Vol. 34, No. 20, pp. 3765–3767, 1985.
Printed in Great Britain.

0006-2952/85 $3.00 + 0.00

SHORT COMMUNICATIONS

The action of VIP on bile secretion and bile acid output in the non-anaesthetized rat

(*Received* 5 *December* 1984; *accepted* 20 *May* 1985)

Vasoactive intestinal polypeptide (VIP) belongs to the "secretin family" of gastrointestinal hormones and is a powerful glycogenolytic agent [1, 2]. Secretin [3, 4] and glucagon [5, 6] which share with VIP many structural similarities, are choleretic in non-anaesthetized rats; this choleresis is associated with an increased biliary transport of bilirubin conjugates [4, 6]. VIP was also described to increase bile secretion in dogs [7], although the functional components of this action were not analysed.

The aim of the present work was to investigate the effect of VIP on bile secretion in rats, and eventually to analyse the mechanism of choleresis. The secretory rate of bile acids into bile was varied to different degrees in order to investigate whether any possible action was mediated by bile acids.

Material and methods

Male Wistar/R rats, 250–300 g body wt, were surgically prepared under pentobarbitone anaesthesia (6 mg/100 g body wt) with catheters in the common bile duct and in the right jugular vein [4] and kept in thermostated boxes for 3 hr to regain and maintain a constant body temperature of 37°. They received 5% glucose in 0.16 M NaCl at the rate of 1.2–1.7 ml/hr/100 g body wt.

Three groups of rats with different rates of bile acid secretion were studied. This was obtained (A) by using rats 3 hr after bile duct cannulation (rats with a relatively unmodified "native" bile acid pool) (VIP, N = 6; saline, N = 6); (B) by a continuous intravenous infusion of 120 nmoles of Na^+–taurocholate/min/100 g body wt ("bile acids supplemented" rats; VIP, N = 7 saline, N = 6); (C) by an 8-hr external biliary drainage whereby bile flow and bile acid secretion rate drop to levels which remain constant in the following hours (bile acid "depleted" rats; VIP, N = 6; saline, N = 7).

After a 30-min collection of control bile, VIP (purchased from G.I.H. Laboratories, Karolinska Institute, Stockholm), dissolved in 0.16 M NaCl was infused over 60 min in the jugular vein at a rate of 300 ng/hr/100 g body wt; followed by a recovery period of 60 min; during the latter period rats received 0.16 M NaCl. Control animals received 0.16 M NaCl instead of VIP. Bile was collected over 10-min periods in tared plastic tubes, on ice and in the dark.

Analytical procedures. Bile flow was measured by gravimetry (weight difference). Bile acids were determined by a 3-alfa-hydroxy-steroid dehydrogenase procedure [8]. Biliary bilirubin was assayed by diazocleavage with ethylanthranilate at pH 2.7 [9] within 30 min after collection of samples. Composition of the bile pigments was analysed by TLC of the methyl ester derivatives of the intact tetrapyrroles after alkaline methanolysis [10].

Results are indicated as mean values ± 1 S.D., unless otherwise specified. The statistically significant difference between basal bile flow and the value under VIP or saline was evaluated by a Student's *t*-test for paired data. Amongst different groups, the test for unpaired data was used.

Results and discussion

The administration of VIP (300 ng/hr/100 g body wt) to rats produces a mild choleresis. This is observed when the secretion rate of bile acids is close to normal (group A; 17% increase), or mildly increased by a continuous infusion of Na^+-taurocholate (group B: 10% increase), but not in rats with a partially depleted bile acid pool (group C) (Table 1). This choleretic effect is far less important than that described in fasting dogs (190%; ref. 7).

The changes produced by VIP were analysed by plotting bile flow (Y axis) as a function of bile acid output (X axis). The intercept with the Y axis, generally considered to represent the so-called bile acid-independent bile flow, was apparently different in the sham and VIP treated animals of groups A and B ($P < 0.01$) (Fig. 1). In group C, the regression line obtained from the control rats ($y = 2.94 + 0.0281x$; $r = 0.542$) was not statistically different (analysis of the covariance) from that describing the VIP treated ones ($y = 3.07 + 0.0304$; $r = 0.586$) nor from that of the whole control group (Fig. 1). From analysing the different components involved in bile secretion, it is obvious that VIP treatment of non-supplemented rats (group A) leads to an increased bile acid independent flow (Table 1) since the bile acid output was unaltered. A similar situation seems present in taurocholate-infused rats (group B). This is also obvious from the observation that bile acid output returned to pretreatment values after discontinuation of VIP whereas the bile flow remained increased at 6.8 μl per min per 100 g body wt for another 60 min. The absence of any effect of VIP in bile depleted animals (group C) could be related to their lower bile flow whereby small changes (of the order of 10–20%) are not obvious due to spreading of individual data or it could be that 8-hr bile depletion induces additional abnormalities obnubiliating possible effects of VIP. Other investigators [11, 12] also documented deviations of the relation between bile flow and bile acid output at low bile acid secretion rates. The mathematical approach of the bile acid independent flow seems thus to hold at normal or high bile acid secretion but not at low output rates. The bile acid independent bile secretion seems enhanced under VIP in analogy with the VIP-regulated mechanism present in the intestine [13] controlling the water and electrolyte secretion by the cell.

In addition to increasing the bile acid independent flow, VIP treatment also enhanced the biliary bile acid excretion rate by 21% in the taurocholate-supplemented animals (group B) (Table 1). The overall bile acid output during the whole VIP-infusion was significantly higher than values of control rats (9275 ± 474 nmoles per 100 g body wt vs 8394 ± 295; $P < 0.05$). This was paralleled by an increased bile flow suggesting that an increased bile acid dependent choleresis is also part of the effects of VIP. The increased bile acid output during VIP in taurocholate infused rats is presumably the result of an increased delivery of bile acids to the liver mediated by an enhanced arterial blood flow to liver and intestine (with ensuing increased portal flow). As the hepatic extraction ratio of bile acids is very high [14], their uptake and output is mainly dependent on hepatic blood flow. Such an effect of VIP will only become apparent when bile acids are given.

Secretin [3, 4] and glucagon [5, 6], two structurally related hormones, have similar choleretic properties in the rat. They are presumably part of a bile flow regulating system, triggered by oral feeding and possibly counteracted in some instances by somatostatin [15] and by substance P

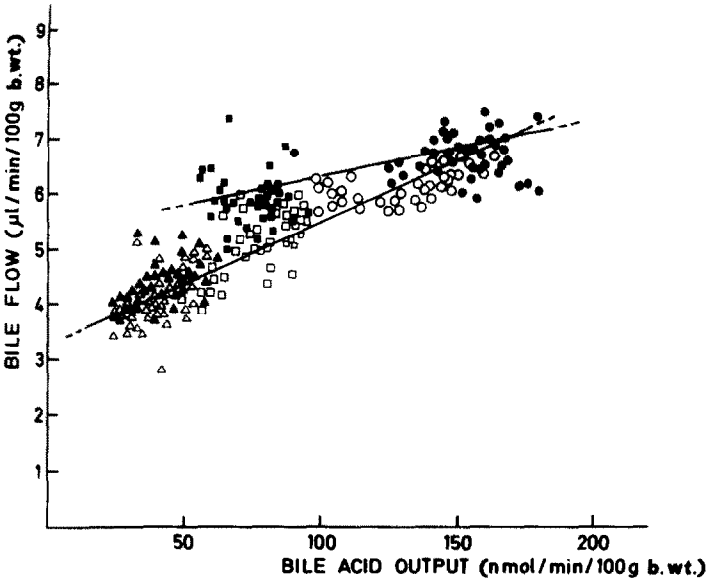

Fig. 1. Relation of bile flow to bile acid output under treatment with NaCl 0.16 M (open symbols) or VIP 300 ng/h/100 g body wt (closed symbols) in six 10-min bile collections obtained from rats (N = 6–7) with bile acid depletion (group C: △, ▲), with a relatively intact native bile acid pool (group A: □, ■) or under Na^+-taurocholate 120 nmol/min/100 g body wt (group B: ○, ●). A single regression line can be fitted to describe the relationship in NaCl treated animals ($y = 3.23 + 0.0225x$; $r = 0.904$; significance against 0 for degrees of freedom N = 17 in consideration of the number of experiments; $P < 0.001$). Under VIP the experimental values can best be fitted into a single regression line when the rats with higher secretory rates (groups A and B) treated with VIP are taken together and separated from group C ($y = 5.31 + 0.0092x$; $r = 0.707$; significance against 0: $P < 0.001$; significance against controls, evaluated by the analysis of covariance for $df_4 = 29$ according to the number of rats: $P < 0.01$).

Table 1. Bile flow (BF) and biliary bilirubin (BR) and bile acid (BA) output (mean ± 1 S.D.) in rats with varied rates of BA secretion, at 30–60 min of an infusion of 0.16 M NaCl (sham treated) or V.I.P. (300 ng/hr/100 g body wt)

			B.F. (μl/min‡)	BR conc. (nmol/l)	BR output (nmol/min*)	BA conc. (nmol/l)	BA output (nmol/min*)
Group A	Sham	(N = 6)	5.1 ± 0.6	104.3 ± 32.2	0.54 ± 0.11	15.2 ± 2.7	77.7 ± 12.4
Native pool	VIP	(N = 6)	6.0 ± 0.3‡	108.7 ± 29.4	0.65 ± 0.13	12.9 ± 2.5	77.4 ± 19.6
Group B	Sham	(N = 6)	6.0 ± 0.3	101.6 ± 29.8	0.63 ± 0.14	21.0 ± 3.2	129.9 ± 19.4
Supplemented†	VIP	(N = 7)	6.8 ± 0.3‡	111.7 ± 35.0	0.76 ± 0.11	23.1 ± 2.1	157.4 ± 9.9‡
Group C	Sham	(N = 7)	4.1 ± 0.5	167.3 ± 37.4	0.69 ± 0.09	10.0 ± 3.3	41.0 ± 9.6
Depleted	VIP	(N = 6)	4.3 ± 0.4	164.8 ± 32.6	0.71 ± 0.13	9.1 ± 2.1	38.8 ± 8.4

* per 100 g body wt.
† Na^+-taurocholate 120 nmol/min/100 g body wt.
‡ $P < 0.05$ as compared to sham rats in the same group.

[16]. The real physiologic regulation awaits for further detailed investigation.

The excretion of endogenous bilirubin was not modified by VIP (Table 1), nor were changes present in the proportion of bilirubin mono- to diglucuronides in bile (not shown), nor in their cumulative (60 min) biliary excretion. This is in line with the absence of any effect of VIP-treatment of rats on their hepatic bilirubin UDP-glucuronyltransferase [17]. In contrast, administration of VIP increased *p*-nitrophenol glucuronyltransferase activity in rat liver [17]. This differential effect seems a further expression of the concept of functional heterogeneity of the transferases [18] as bilirubin and *p*-nitrophenol belong to a different group of substrates.

In summary, VIP has a mild choleretic action (10–17%) in rats. This effect is only observed in the presence of a normal or high bile acid secretion and not in bile acid depleted rats. VIP-induced choleresis results both from an enhanced bile acid independent and a bile acid dependent flow, as the bile acid output of rats receiving Na^+-taurocholate intravenously increased 21%.

Acknowledgements—Authors are indebted to Mrs Van Houtte for help with the animal experiments. The work was supported by the Foundation for Scientific Medical Research of Belgium.

Laboratory of Hepatology
Department of Medical Research
Catholic University of Leuven
B-3000 Leuven, Belgium, and
**Laboratorio di Biochimica Gastroenterologica ed Epatologica (LA.BI.GE.E.)*
Cattedra di Gastroenterologia del' Universitá di Roma "La Sapienza"
00161 Italy

GIOVANNI L. RICCI*
JOHAN FEVERY

REFERENCES

1. P. J. Magistretti, J. H. Morrison, W. J. Shoemaker, V. Sapin and F. E. Bloom, *Proc. natn. Acad. Sci. U.S.A.* **78**, 6535 (1981).
2. C. Kerins and S. I. Said, *Proc. Soc. exp. Biol. Med.* **142**, 1014 (1973).
3. C. Balabaud, M. Noel and J. Dangomau, *J. Pharmac. (Paris)*, **8**, 191 (1977).
4. G. L. Ricci, R. Michiels, J. Fevery and J. De Groote, *Hepatology* **4**, 651 (1984).
5. C. Balabaud, D. Peron-Marc, M. Noel, and J. Dangomau, *J. Pharmac. (Paris)* **7**, 265 (1976).
6. G. L. Ricci, R. Michiels, J. Fevery, J. De Groote and K. P. M. Heirwegh, *Gastroenterology* **84**, 1393(A) (1983).
7. L. Thulin and M. Hellgreen, *Acta chir. scand.* **142**, 235 (1976).
8. S. D. Turley and J. M. Dietschy, *J. Lipid Res.* **19**, 924 (1978).
9. F. P. Van Roy and K. P. M. Heirwegh, *Biochem J.* **107**, 507 (1968).
10. N. Blanckaert, *Biochem J.* **185**, 115 (1980).
11. C. Balabaud, K. A. Kron and J. J. Gumucio, *J. Lab. Clin. Med.* **89**, 393 (1977).
12. A. L. Baker, R. A. B. Wood, A. R. Moossa and J. L. Boyer, *J. clin. Invest.* **64**, 312 (1979).
13. I. M. Coupar, *Clin. Exp. Pharmac. Physiol.* **3**, 615 (1976).
14. N. B. Javitt, *N. Engl. J. Med.* **295**, 1464 (1976).
15. G. L. Ricci and J. Fevery, *Gastroenterology* **81**, 552 (1981).
16. I. Magnusson and L. Thulin, *Acta physiol. scand.* **160**, 293 (1979).
17. G. L. Ricci, R. Michiels and K. P. M. Heirwegh, *Clin. Sci.* **64**, 53P (1983).
18. G. J. Wishart and M. T. Campbell, *Biochem. J.* **178**, 443 (1979).

Biochemical Pharmacology, Vol. 34, No. 20, pp. 3767–3771, 1985.
Printed in Great Britain.

0006-2952/85 $3.00 + 0.00

Effect of plasma on human erythrocyte *beta*-adrenergic receptors

(*Received* 26 *November* 1984; *accepted* 28 *May* 1985)

Beta-adrenergic receptors were until recently considered absent on human erythrocytes. Catecholamine sensitive adenylate cyclase activity was found to be extremely low in the human red cell membrane [1–5] and *beta*-adrenergic receptor binding sites were not detectable [6, 7] until recent observations [8–10] showed a small, but reproducible number of binding sites for *beta*-adrenergic ligands.

The binding of the *beta*-adrenergic blockers (±)-propranolol and (−)-alprenolol to intact human erythrocytes involved saturable and non saturable binding [11]. Using a model with one class of saturable binding sites, it was found that each cell contained approximately 6000 high affinity binding sites in whole blood. By washing the number of binding sites was reduced to approximately 600 per cell, but after resuspension of washed cells in autologous plasma, the binding capacity became similar to that of erythrocytes in whole blood. These results indicated that the saturable, but not the non saturable binding to human erythrocytes was influenced by the presence of plasma and by washing.

The present study was undertaken for further characterization of the influence of autologous plasma and washing on binding capacity for propranolol and to determine whether plasma influences the catecholamine sensitive adenylate cyclase through these binding sites.

Materials and methods

The following chemicals were employed in the study: [^{3}H]-(−)-propranolol (spec. act. 19.6 Ci/mmol), [^{3}H]-adenosine 3′,5′-cyclic monophosphate (spec. act. 37 Ci/mmol) New England Nuclear, Dreieich, F.R.G.; unlabelled (−)-propranolol from Radiochemical and Pharmaceutical Division of Imperial Chemical Industries Ltd., London, U.K.; (−)-isoproterenol hydrochloride, theophylline and adenosine 3′,5′-cyclic monophosphate from Sigma Chemical Corp., St. Louis, MI.; sodium heparin from NOVO industries, Copenhagen, Denmark. Other chemicals were of analytical grade.

Washing and incubation buffer (modified Krebs Ringer phosphate buffer): NaCl 122 mM, KCl 4.9 mM, $MgSO_4$ 1.2 mM, $CaCl_2$ 1.3 mM, Na_2HPO_4 15.9 mM, d-glucose 10 mM, heparin 10 IU/ml. pH was adjusted to 7.40.

Blood was obtained from young, healthy individuals without any medication after an overnight fast and collected in plain glass tubes containing heparin to achieve a final concentration of 10 IU/ml. Whole blood was gassed with 5% CO_2 in air to achieve pH 7.4. The erythrocytes were separated from plasma and buffer by centrifugation at 1000 *g* for 15 min and plasma including buffy coat was aspirated. After one, four and nine washes with buffer at 22°, the cells were resuspended in the buffer at pH 7.4. The original hematocrit was maintained during the washing and in the final resuspension.

Platelet poor plasma was obtained by centrifugation of plasma for 20 min at 105,000 *g*. Carbon dioxide was partially removed from the plasma by gentle stirring at 22° for 3 hr and pH was adjusted to 7.4 with 0.1 M HCl.

The binding of (−)-propranolol to cells was determined by simultaneous addition of labelled and unlabelled ligand to achieve unbound concentrations from 0.1 nM to 100 μM in 2 ml of whole blood or in cell suspensions with original hematocrit. After 1 hr at 22°, the cells were separated from the incubation medium (plasma or buffer) by a centrifugation assay described before [11]. (−)-Propranolol binding to the erythrocytes was calculated from the equation

$$R = T \times (1/H) - P \times (1-H)/H,$$

T representing total radioligand concentration in the incubation mixture, R and P being the radioligand concentration in erythrocytes and supernatant (plasma or buffer) and H being the hematocrit. From the distribution of radioligand and the ratio between labelled and unlabelled ligand, the total (−)-propranolol binding to erythrocytes was calculated. Non saturable binding (B_{NS}) to the