Table 2. Circadian variation in B_{max}, K_D and platelet count. Results from cosinor analysis with a period $\tau = 24$ hr, and data expressed as % of individual 24-hr mean (M) in order to minimize interindividual differences in 24-hr means

Variable	No. of data	P*	Double-amplitude (% M)†	Acrophase (hr., min)‡ (95% confidence limits)
B_{max}	28	0.01	19 (3; 34)	0350 (0015; 0800)
K_D	28	0.99	—	—
Platelet number	28	0.003	17 (5; 29)	1100 (0800; 1410)

* P-value from an F-test of the rejection of the null-amplitude hypothesis.
† Difference between values at maximum and minimum in fitted cosine function with $\tau = 24$ hr.
‡ Location of the maximum in fitted cosine function, referred to midnight.

binding sites in human platelets exhibits a circadian rhythm in Man. Such a predictable variation along the 24-hr time scale must be taken into account in further investigations of the physiological role and regulation of such peripheral benzodiazepine binding sites.

In summary, a circadian rhythm in peripheral type benzodiazepine binding sites is described and statistically validated in platelets from peripheral blood of healthy human volunteers. Maximum values in B_{max} are observed near the middle of the night and the peak–trough difference equals 20% of the 24-hr mean.

*C.N.R.S. UA 581
Chronobiologie et Chronopharmacologie
Fondation Adolphe de Rothschild
29, rue Manin
75940 Paris Cedex 19
France

†Pharmuka Groupe Rhône-Poulenc
35 Quai du Moulin de Cage
92231-Gennevilliers
France

FRANCIS LEVI*
JESUS BENAVIDES†
YVAN TOUITOU*
DOMINIQUE QUARTERRONET†
THIERRY CANTON†
ANDRÉ UZAN†
CLAUDE GUEREMY†
GÉRARD LE FUR†
ALAIN REINBERG*

REFERENCES

1. C. Baestrup and R. F. Squires, *Proc. natn. Acad. Sci. U.S.A.* 3805 (1977).
2. J. W. Regan, H. J. Yamamura, S. Yamada and W. R. Roeske, *Life Sci.* **30**, 991, (1981).
3. H. Schoemaker, R. G. Boles, W. O. Horst and H. I. Yamamura, *J. Pharmac. exp. Ther.* **225**, 61 (1983).
4. J. K. T. Wang, T. Taniguchi and S. Spector, *Molec. Pharmac.* **25**, 349 (1984).
5. G. Le Fur, M. L. Perrier, N. Vaucker, F. Imbault, A. Flamier, J. Benavides, A. Uzan, C. Renault, M. C. Dubroeucq, and C. Guerremy, *Life Sci.* **32**, 1839 (1983).
6. G. Le Fur, F. Guilloux, P. Rufat, J. Benavides, A. Uzan, C. Renault, M. C. Dubroeucq and C. Gueremy, *Life Sci.* **32**, 1849 (1983).
7. C. Le Fur, N. Vaucher, M. L. Perrier, A. Flamier, J. Benavides, C. Renault, M. C. Dubroeucq, C. Gueremy and A. Uzan, *Life Sci.* **33**, 449 (1983).
8. M. Mestre, T. Carriot, C. Belin, A. Uzan, C. Renault, M. C. Dubroeucq, C. Gueremy and G. Le Fur, *Life Sci.* **35**, 953 (1984).
9. M. Mestre, T. Carriot, C. Belin, A. Uzan, C. Renault, M. C. Dubroeucq, C. Guérémy, A. Doble and G. Le Fur, *Life Sci.* **36**, 391 (1985).
10. J. Benavides, F. Guilloux, D. E. Allam, A. Uzan, J. Mizoule, C. Renault, M. C. Dubrouecq, C. Gueremy and G. Le Fur, *Life Sci.* **34**, 2613 (1984).
11. J. Mizoule, A. Gauthier, A. Uzan, C. Renault, M. C. Dubroeucq, C. Gueremy and G. Le Fur, *Life Sci.* (in press).
12. J. Benavides, P. Quarteronet, P. F. Plouin, F. Imbault, T. Phan, A. Uzan, C. Renault, M. C. Dubrouecq, C. Gueremy and G. Le Fur, *Biochem. Pharmac.* **33**, 2467 (1984).
13. M. S. Kafka, A. Wirz-Justice and D. Naber, *Brain Res.* **207**, 409 (1981).
14. A. Wirz-Justice, I. Tobler, M. S. Kafka, D. Naber, P. Marangos, A. Borbelly and T. Wehr, *Psychiatry Res.* **5**, 67 (1981).
15. D. Naber, A. Wirz-Justice and M. S. Kafka, *Neurosci. Lett.* **2**, 45 (1981).
16. A. Wirz-Justice, K. Kräuchi, T. Morimasa, R. Willever and H. Feer, *Eur. J. Pharmac.* **87**, 331 (1983).
17. E. K. Perry, R. H. Perry and B. E. Tomlinson, *Neurosci. Lett.* **4**, 185 (1977).
18. E. C. Rossi, *J. Lab. clin. Med.* **78**, 483 (1971).
19. O. H. Lowry, N. J. Rosebrough, A. C. Farr and R. J. Randall, *J. biol. Chem.* **193**, 265 (1951).
20. J. A. Zivin and D. R. Waud, *Life Sci.* **30**, 1407 (1982).
21. W. Nelson, Y. L. Tong and F. Halberg, *Chronobiologia* **6**, 305 (1979).

* To whom correspondence should be sent.

Biochemical Pharmacology, Vol. 35, No. 15, pp. 2625–2628, 1986.
Printed in Great Britain.

0006-2952/86 $3.00 + 0.00
Pergamon Journals Ltd.

Bile flow decrease and altered bile composition in streptozotocin-treated rats

(Received 14 *August* 1985; *accepted* 23 *December* 1985)

Streptozotocin (SZ), an antibiotic produced by *Streptomyces achromogenes*, is a potent inducer of diabetes in laboratory animals, attributable to the irreversible damage which it exerts, especially on the β-cells [1]. SZ distribution in tissues indicates the important role played by the liver in the metabolism and excretion of this compound [2]. Previous studies from this laboratory demonstrated that SZ administration to rats induced a transient impairment of hepatobiliary function [3]. In the present study, we investigated the effect of SZ treatment of rats on the bile-acid-dependent flow (BADF) and the bile-acid-independent flow (BAIF) of bile. In addition, the permeability of the biliary system to sucrose and the biliary secretion of some endogenous bile components were also investigated.

Materials and methods

Adult male Wistar rats weighing 310–370 g were used. SZ (Sigma Chemical Co., U.S.A.) was dissolved in 0.05 M citrate buffer, pH 4.5 [4], and injected i.v. as a single dose of 50 mg/kg body wt. Control rats were injected with buffer alone. All the experiments were performed 24 hr after the injection. The animals were anesthetized with sodium pentobarbital (50 mg/kg body wt, i.p.), and the bile duct, the femoral vein and the femoral artery were cannulated with appropriate polyethylene catheters. Rectal temperature was monitored continuously in all the rats and maintained at 38.0 ± 0.5° throughout the experiments to prevent hypothermic alterations of bile flow (BF), as stated elsewhere [5]. A venous blood sample (50 μl) was systematically obtained from all the rats for serum glucose determination [6] (GOD-PAP test, Boehringer Mannheim, F.R.G.).

Immediately after cannulation the bile was collected for 60 min (basal period). An i.v. sodium taurocholate (TC) (Sigma Chemical Co., U.S.A.) infusion was started after the basal period of bile collection. TC was dissolved in phosphate buffer (pH 7.4) and given at the rate of 300 nmoles/min/100 g body wt (8.34 μl/min) [7]. After the beginning of the infusion, four bile samples were collected every 15 min. Then, the liver was removed and weighed.

BF (gravimetry), and the concentrations of bile acids (BA) [8], cholesterol (Cho) (Colestat, Wiener Lab, Argentina), phospholipids (PL) [9], and protein (Pr) [10], together with the activity of lysosomal acid phosphatase (AP) (FAcP test, Wiener Lab, Argentina), were determined in all the bile samples. The Cho saturation index of bile was also calculated [11], and the solubility limit of Cho was chosen as established previously [12]. BAIF was estimated by extrapolation for zero BA secretion of the regression line between BF and BA secretion rate [13].

In another set of experiments, the bile-to-plasma concentration ratio (B/P) of sucrose was used to estimate the permeability of the biliary system [14]. For this purpose, [^{14}C]sucrose (2 μCi, sp. act. 360 mCi/mmole) (ICN, U.S.A.) was injected i.v. without ligating the renal pedicles [15]. Bile samples were collected from 30–60, 60–90 and 90–120 min after the sucrose injection. Arterial blood samples (200 μl) were taken at the mid-point of each bile collection period. Clearance of sucrose was calculated as the product of BF times the bile-to-plasma ratio of ^{14}C-activity.

Animals from another group were anesthetized, blood was withdrawn by heart puncture, and the liver was removed promptly. The largest lobe of the liver was homogenized in 9 vol. of ice-chilled Sörensen buffer (pH 7.4), and the homogenate was centrifuged in a refrigerated centrifuge (3000 rpm, 10 min). The supernatant fraction was used for determination of Cho, PL, Pr [16] and AP.

The unpaired *t*-test was used for comparison between groups, and the paired *t*-test for within group comparisons; the level of significance was chosen as $P < 0.05$. The data were the means ± S.E.M.

Results

As expected, serum glucose levels were increased markedly in SZ-treated rats (4.8 ± 0.1 g/l, N = 9) compared with controls (1.2 ± 0.1 g/l, N = 9).

Data on BF and biliary lipid output during the basal period of bile collection and following the infusion of TC are presented in Table 1. BF and BA output were diminished significantly in treated animals during the basal period of bile collection, whereas the outputs of Cho and PL and the Cho saturation index were increased. The BF and the BA output increased significantly in control and treated rats during TC infusion. The outputs of Cho and PL were increased significantly by TC in control rats; the values obtained in treated animals during the infusion did not differ statistically from basal values. However, for all the variables tested during the infusion, the increases over

Table 1. Bile flow and biliary lipid output in control and SZ-treated rats during the basal period and following the infusion of TC

	BF (μl/min/g liver)	BA output (nmoles/min/g liver)	Cho output (nmoles/min/g liver)	PL output (nmoles P/min/g liver)	Cho saturation index
Control rats					
Basal period	2.3 ± 0.1	55.9 ± 2.4	1.2 ± 0.1	9.7 ± 1.2	0.36 ± 0.03
TC infusion period	2.7 ± 0.1*	87.8 ± 4.0*	1.6 ± 0.2*	16.8 ± 1.8*	0.32 ± 0.04
Increase from basal value	0.4 ± 0.1	31.8 ± 4.7	0.4 ± 0.1	7.3 ± 2.6	
SZ-treated rats					
Basal period	1.6 ± 0.1†	34.2 ± 3.3†	2.1 ± 0.3†	13.4 ± 1.2†	0.53 ± 0.05†
TC infusion period	2.0 ± 0.1*	63.1 ± 6.9*	2.5 ± 0.3	19.6 ± 2.3	0.50 ± 0.1
Increase from basal value	0.5 ± 0.1	33.4 ± 5.2	0.5 ± 0.3	6.2 ± 3.2	

Bile was collected for 60 min immediately after cannulation (basal period). After this period, an i.v. infusion of TC was started and maintained for 60 min (TC infusion period). Data are means ± S.E.M. from five to nine rats. Increases from basal values refer to the values obtained during the infusion period minus the respective values of the basal period of bile collection (means ± S.E.M.).

* Significantly different from the values obtained for the respective basal period of bile collection.

† Significantly different from controls.

baseline induced by TC in both groups seemed comparable. Furthermore, the recovery of infused TC (calculated as moles of bile salt excreted over basal values after 60 min of infusion and related to the amount of TC infused during this period) was similar in both groups (controls, 34.0 ± 4.4%, N = 9; SZ-treated, 38.6 ± 6.1%, N = 9).

The outputs of Pr and AP during the basal period of bile collection were diminished significantly in SZ-treated rats in comparison with controls. Figure 1 shows that during the infusion of TC the excretory patterns of Pr and AP were different in both groups. Such a response was clearly seen for the AP excretory rate which rose during the infusion period to values significantly higher than controls.

The relationship between BF and BA secretion rate in both groups is shown in Fig. 2. It can be seen that BAIF was diminished in SZ-treated rats, whereas the slope of the regression line between BF and BA secretion was similar in both groups.

The B/P for sucrose did not show any difference between groups (controls, 0.23 ± 0.02, N = 4; SZ-treated, 0.26 ± 0.04, N = 3); consequently, sucrose biliary clearance decreased in parallel with BF in treated rats (controls, 0.52 ± 0.07 μl/min/g liver; SZ-treated, 0.36 ± 0.10 μl/min/g liver).

On the other hand, no differences between groups were found in liver Pr (controls, 164.1 ± 5.9 mg/g liver, N = 4; SZ-treated, 171.8 ± 7.1 mg/g liver, N = 3) and PL (controls, 1212.7 ± 27.8 μmoles P/g liver, N = 4; SZ-treated, 1210.2 ± 22.3 μmoles P/g liver, N = 4). Conversely, AP activity and Cho concentration in the supernatant fraction were significantly greater in SZ-treated rats than in controls (AP activity: controls, 2183.3 ± 56.7 mU/g liver, N = 4; SZ-treated, 2588 ± 91.0 mU/g liver, N = 3; and Cho: controls, 0.36 ± 0.07 μmole/g liver, N = 4; SZ-treated, 3.13 ± 1.09 μmoles/g liver, N = 4).

Discussion

The results of this study show that SZ produced in rats a decrease in BF (−30%) related to a decrease in BAIF (−37%) and probably in BADF because the output of BA was also diminished in SZ-treated rats by 39%. Sodium transport linked to (Na^+–K^+)ATPase [17] and bicarbonate [18] have been implicated in BAIF generation. In this connection, it was reported that different cholestatic drugs would act by decreasing (Na^+–K^+)ATPase activity with subsequent diminution of BAIF [19]. It was also assumed that the decrease in BAIF was of canalicular origin because the ductular component of biliary secretion in the rat has a negligible contribution to bile production [20]. In addition, the permeability of the biliary system estimated in this study by B/P of sucrose was not affected by SZ treatment.

The decrease in BADF was consistent with the diminished BA output observed in SZ-treated rats (see Table 1). Since the recovery of infused TC was found to be similar

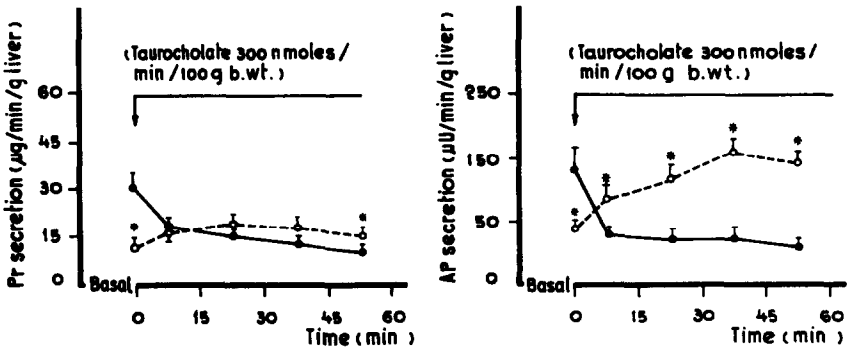

Fig. 1. Pattterns of Pr and AP outputs during the basal period of bile collection and following the infusion of TC. Data are means ± S.E.M. for four rats of each group. Key: (●—●) control rats; and (○---○) SZ-treated rats. The asterisks indicate significant differences between groups. Arrows signal starting of TC infusion.

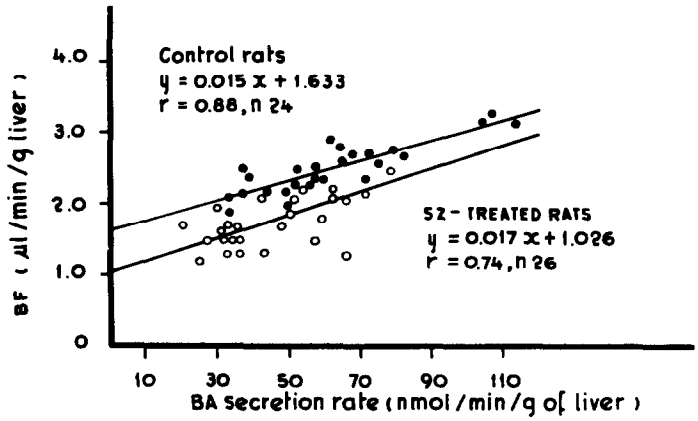

Fig. 2. Relationship between BF and BA secretion rate. Data are individual values obtained from control (●) and SZ-treated rats (○).

in control and treated rats, it may be suggested that BA hepatic transport was not affected by SZ. Impaired BA output in treated rats might be also a result of an alteration of the enterohepatic circulation of BA produced either by a direct effect of SZ on the intestinal cells or by the antibiotic activity of SZ on the intestinal bacteria involved in bile salt biotransformation [21]. In this connection, some antibiotics may affect the enterohepatic circulation of xenobiotics [22].

Increases in hepatic and biliary Cho in treated rats may be a consequence of decreased levels of insulin [23] induced by a diabetogenic dose of SZ, and the increase in PL biliary output (see Table 1) might be associated with that of Cho [24].

As expected [24], the infusion of TC produced increased outputs of Cho and PL in control rats though the response appeared less effective in treated animals (see Table 1).

An additional observation of interest was the decrease in the outputs of Pr and AP in SZ-treated rats and their increase during the infusion of TC in these animals (see Fig. 1). It is probably the role of BA to favor the uptake and intrahepatic transport of Pr [25] and the biliary secretion of lysosomal vesicles including enzymes like AP.* Therefore, the decrease in BA secretion produced by SZ might be involved in the decreases in Pr and AP outputs, because SZ did not affect either hepatic Pr or serum Pr (control 7.0 ± 0.2 g/100 ml, N = 4; SZ-treated, 6.7 ± 0.3 g/100 ml, N = 4). Since AP activity was increased in the livers of SZ-treated rats, it is understandable that the lysosomal enzyme accumulated may be released into bile by TC to a greater extent than Pr which, however, reached the bile levels seen in the controls.

In conclusion, SZ produced a decrease in BF at the expense of both BAIF and BADF without impairing the permeability of the biliary system. In addition, this diabetogenic compound altered the biliary secretion of lipid and protein components though the primary effect remains to be clarified. The effects induced by SZ should be considered in hepatic metabolism studies in experimental diabetes induced shortly after the administration of this compound.

Acknowledgements—This work was supported by a research grant from the Consejo Nacional de Investigaciones Científicas y Técnicas (CONICET), República Argentina. The valuable technical assistance of Mr. Raúl Trbojevich is gratefully acknowledged.

* R. A. Marinelli, M. G. Luquita and E. A. Rodríguez Garay, manuscript in preparation.

† Address correspondence to: Dr. Emilio A. Rodríguez Garay, Instituto de Fisiología Experimental, Facultad de Ciencias Bioquímicas y Farmacéuticas, Universidad Nacional de Rosario, Suipacha 531, 2000 Rosario, Argentina.

Instituto de Fisiología Experimental
Consejo Nacional de Investigaciones Científicas y Técnicas (CONICET)
Universidad Nacional de Rosario
Rosario, Argentina

CRISTINA E. CARNOVALE
RAÚL A. MARINELLI
E. A. RODRÍGUEZ GARAY†

REFERENCES

1. L. M. Srivastava, P. S. Bora and S. D. Bhatt, *Trends pharmac. Sci.* **3**, 376 (1982).
2. P. Masiello, E. H. Karunanayake, E. Bergamini, D. J. Hearse and G. Mellows, *Biochem. Pharmac.* **30**, 1907 (1981).
3. C. Carnovale and E. A. Rodríguez Garay, *Experientia* **40**, 248 (1984).
4. R. Herr, T. Bergy and H. Jahnke, *Antibiotics A.* 236 (1956–1960).
5. J. Roberts, C. Klaassen and G. Plaa, *Proc. Soc. exp. Biol. Med.* **125**, 313 (1967).
6. P. Trinder, *Ann. clin. Biochem.* **6**, 24 (1969).
7. S. Erlinger, D. Dhumeaux, J. P. Benhamou and R. Fauvert, *Revue fr. Étud. clin. Biol.* **14**, 144 (1969).
8. P. Berthelot, S. Erlinger, D. Dhumeaux and A. Preaux, *Am. J. Physiol.* **219**, 809 (1907).
9. G. R. Bartlett, *J. biol. Chem.* **234**, 466 (1959).
10. O. H. Lowry, N. J. Rosebrough, A. L. Farr and R. J. Randall, *J. biol. Chem.* **193**, 265 (1951).
11. P. J. Thomas and A. F. Hoffmann, *Gastroenterology* **65**, 698 (1973).
12. R. T. Holzbach, M. Marsh, M. Olszewski and K. Holan, *J. clin. Invest.* **52**, 1467 (1973).
13. S. Erlinger and D. Dhumeaux, *Gastroenterology* **66**, 281 (1974).
14. E. L. Korker, *J. clin. Invest.* **46**, 1189 (1967).
15. C. D. Klaassen, *J. Pharmac. exp. Ther.* **176**, 743 (1971).
16. E. Suranyi and Y. Avi-Dor, *Biochim. biophys. Acta* **118**, 445 (1966).
17. J. L. Boyer, *Physiol. Rev.* **60**, 303 (1980).
18. W. G. M. Hardison and C. A. Wood, *Am. J. Physiol.* **235**, E158 (1978).
19. J. Reichen and G. Paumgartner, *J. clin. Invest.* **60**, 429 (1977).
20. E. L. Forker, *A. Rev. Physiol.* **39**, 323 (1977).
21. H. P. A. Illing, *Xenobiotica* **11**, 815 (1981).
22. D. J. Back, A. M. Breckenridge, F. E. Crawford, K. J. Cross, M. Orme, A. Percival and P. Rowe, *J. Steroid Biochem.* **13**, 95 (1980).
23. W. C. Meyers and R. S. Jones, *Am. J. Surg.* **137**, 7 (1979).
24. F. Nervi, R. del Pozo, C. F. Covarrubias and B. O. Ronco, *Hepatology* **3**, 360 (1983).
25. A. L. Jones, D. L. Schmucker, R. H. Renston and T. Murakami, *Digestive Dis. Sci.* **25**, 609 (1980).

Biochemical Pharmacology, Vol. 35, No. 15, pp. 2628–2630, 1986.
Printed in Great Britain.

0006-2952/86 $3.00 + 0.00
Pergamon Journals Ltd.

Age-associated alteration in imipramine metabolism is position selective

(*Received* 10 *December* 1985; *accepted* 3 *March* 1986)

Imipramine is one of the widely prescribed tricyclic antidepressants. It is extensively metabolized by the hepatic microsomal cytochrome P-450 and forms 2-hydroxy imipramine and desipramine as primary metabolites by 2-hydroxylation and *N*-demethylation, respectively. In our previous study [1], we showed that the imipramine *N*-demethylase activity was about six times higher in 3-month-old male Wistar rats than in females of the corresponding age ($P < 0.01$). In contrast, there was little sex difference in imipramine 2-hydroxylase activity. These observations supported the hypothesis that these two metabolic pathways are mediated in large part by different species of