Biochemical Pharmacology, Vol. 37, No. 21, pp. 4129–4134, 1988.
Printed in Great Britain.

0006-2952/88 $3.00 + 0.00

EFFECTS OF CHLORPROMAZINE ON TAUROCHOLATE TRANSPORT IN ISOLATED RAT HEPATOCYTES

NEILL H. STACEY
National Institute of Occupational Health and Safety, The University of Sydney, N.S.W. 2006, Australia

(*Received* 8 *February* 1988; *accepted* 20 *May* 1988)

Abstract—Chlorpromazine has been shown to have no effect on the uptake of the endogenous bile salt substrate, taurocholate, by isolated rat hepatocytes. It has been shown, however, to inhibit directly release of taurocholate from pre-loaded cells over extended incubation. However, there was no inhibition of the efflux process *per se* as shown by similar initial rates of taurocholate efflux in the presence or absence of chlorpromazine. Pretreatment of rats with chlorpromazine (100 μmoles/kg) resulted in no change in the ability to transport (that is, accumulate or secrete) taurocholate by hepatocytes isolated 2, 24, 36, 48, or 60 hr later. The data indicate that, if a direct effect on bile acid transport is important in chlorpromazine induced biliary dysfunction, then it involves release rather than uptake at the cell membrane. However, as efflux itself is not inhibited chlorpromazine may interfere with release of taurocholate from intracellular sites.

Use of the psychotropic drug, chlorpromazine, has resulted in a reported incidence of clinical jaundice of about 1%. Subclinical interference with biliary function may be as high as 50% although caution has been advised in using this figure [1]. Side effects of chlorpromazine remain a significant problem as it was found to account for almost 10% of drug-associated hepatotoxicity in a 10 year Danish study and some 10% of these had a fatal outcome [2].

One of the major problems in studying a drug such as chlorpromazine is the non-predictable nature of the adverse response. Seemingly linked with this has been the inability to provide a representative animal model of the injury [1]. The mechanism by which biliary dysfunction occurs is so unclear that it has been suggested that it may be due to a combination of several factors [3, 4].

Recent experimental evidence has led to the suggestion that chlorpromazine may, in fact, be directly hepatotoxic after all [4, 5]. Other studies have also reported direct effects of chlorpromazine on biliary function and these have been used to support the view that the clinical hepatotoxic response to chlorpromazine is also of a direct nature [6]. These authors concluded from a study using isolated perfused rat liver that chlorpromazine caused an impairment of the uptake and eventual excretion of bile acids. More recently it has been shown that acutely administered chlorpromazine to rats results in interference with transport of indocyanine green [7] and horseradish peroxidase [8].

It is generally accepted that an inhibition of biliary function could be caused by an interference with the uptake of bile acids into the hepatocyte, movement across the cell or excretion from it [4, 9]. Suspensions of isolated hepatocytes have been used to study the interference by chemicals with the uptake and/or excretion of model substrates. The approaches taken include pretreatment of the animal [10–15] and direct addition to the cell suspension [15–18].

The current study was therefore undertaken to determine, at the cellular level, to what extent chlorpromazine interferes with the uptake and excretion of taurocholate in isolated hepatocytes both directly and after animal pretreatment.

MATERIALS AND METHODS

Tauro[carbonyl-^{14}C]cholic acid, sodium salt (60 mCi/mmol) was purchased from Amersham (Sydney) while sodium taurocholate and chlorpromazine HCl were obtained from Sigma (St Louis, MO). Collagenase was Worthington CLS II. All other chemicals used were reagent grade from local agents.

Sprague–Dawley rats (260–320 g) from the University of Sydney Animal House were used as liver donors. They were allowed free access to food (Allied Stock Feeds, Sydney) and water. Some rats were pretreated intraperitoneally with chlorpromazine (100 μmoles/kg) 2, 24, 36, 48 or 60 hr before hepatocyte isolation. Ether was used as the anaesthetic and surgery was performed at about 0930 for each experiment. Hepatocytes were isolated by the method of Berry and Friend [19] with minor modifications as previously described [20]. Hepatocytes were resuspended to 25 mg wet weight cells/ml (determined by weighing the freshly isolated cell pellet with allowance for intercellular fluid) and initial viability determined by trypan blue exclusion was approximately 85%. Potassium ion concentration was also used to assess viability.

Incubation medium was a Tris-buffered balanced salt solution (131 mM NaCl, 5.2 mM KCl, 0.9 mM $MgSO_4$, 1 mM $CaCl_2$, 3 mM Na_2HPO_4, 10 mM Tris (hydroxymethyl) aminomethane, pH 7.4). For determination of effects on uptake, suspensions of freshly isolated hepatocytes (2 ml) were first preincubated (37° with shaking at 80 oscillations/min) for 15 min with or without chlorpromazine at a final con-

BP 37:21-G

centration of 1, 5, 10, 50 or 100 μM. Following pre-incubation, 80 μl substrate (^{14}C taurocholate, 80 nCi/ml) was added at a final concentration of 25 μM and uptake determined at appropriate times. Sampling was carried out by the silicone oil centrifugation technique as previously described [21]. The samples containing ^{14}C were placed in scintillation fluid after the cell pellet was dissolved in the potassium hydroxide and were quantitated in a Packard liquid scintillation counter. Determination of the amount of radiolabel and protein concentration [22] allowed calculation of uptake per milligram of protein. Allowance for adherent fluid was made in the calculations as documented by Eaton and Klaassen [23].

In efflux studies, hepatocytes were pre-loaded with ^{14}C-taurocholate by incubation of 350 mg cells/2 ml for 20 min with final concentrations of 5–100 μM at 160 nCi/ml of suspension. Subsequently an aliquot (100 μl) of this concentrated cell suspension was added to 1.9 ml fresh incubation medium which contained chlorpromazine or saline vehicle (15 min pre-incubation). One set of experiments was performed to determine taurocholate release at intervals over a 30 min incubation period. A second series (in untreated rats) at varying preloading concentrations was carried out to investigate efflux in the linear range (sampling at 30, 60, 90, 120, 150 and 180 sec). Samples were then taken for determination of cellular taurocholate content as described and referenced for the uptake studies.

Endogenous levels of taurocholate in hepatocytes isolated from control and 48 hr pretreated rats were assayed by HPLC after methanol extraction (1:1) of cells [24].

Statistical analyses were by analysis of variance and Duncan's test or by *t*-test with a pre-set probability level of $P < 0.05$. Each data point represents a mean value obtained from at least three, but generally four, experiments, with each experiment using hepatocytes isolated from a different rat.

RESULTS

Chlorpromazine at concentrations up to 100 μM was found to have no effect on uptake of taurocholate over a 30 min incubation period (Fig. 1). Data for the other chlorpromazine concentrations (1, 5, 10 and 50 μM) are not shown as these were also not different to the controls. The data for release of taurocholate over a 30 min incubation period are expressed as the difference between the value at the particular sample time and the value at 1 min (Table 1). It can be seen that chlorpromazine at 50 and 100 μM causes an inhibition of efflux at all sampling times of 5 min or greater (except 30 min–100 μM). A second series of experiments was carried out to examine efflux of taurocholate in the linear range (30–180 sec) with and without 100 μM chlorpromazine. The initial velocity of efflux (Voe) was determined as the slope of the lines for each pre-loading concentration of taurocholate. No difference in response to chlorpromazine was found (Table 2).

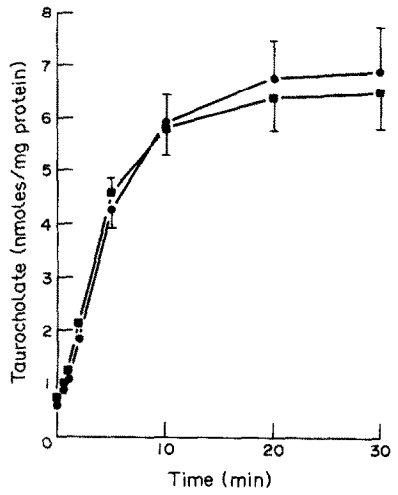

Fig. 1. Effects of chlorpromazine (100 μM) added directly to the incubation vessel on uptake of taurocholate by isolated rat hepatocytes. Points represent means and bars standard errors. Some SE are omitted at the early time points for the sake of clarity.

After rat pretreatment with chlorpromazine sub-

Table 1. Effect of chlorpromazine on release of taurocholate from hepatocytes over 30 min

[Chlorpromazine] μM	Sample time (min) 2	5	10	20	30
0	0.38 ± 0.12[a]	1.29 ± 0.18	1.91 ± 0.26	2.19 ± 0.32	2.19 ± 0.25
1	0.45 ± 0.18	1.23 ± 0.22	1.92 ± 0.32	2.20 ± 0.45	2.24 ± 0.56
5	0.23 ± 0.05	0.85 ± 0.09	1.64 ± 0.14	1.89 ± 0.24	1.97 ± 0.34
10	0.30 ± 0.07	1.15 ± 0.15	1.89 ± 0.17	1.73 ± 0.34	2.13 ± 0.38
50	0.17 ± 0.14	0.66 ± 0.12[b]	1.00 ± 0.07[b]	1.15 ± 0.10[b]	1.11 ± 0.22[b]
100	0.30 ± 0.07	0.54 ± 0.18[b]	0.97 ± 0.06[b]	1.12 ± 0.10[b]	1.33 ± 0.13

[a] Values are taken from the difference between the sample at 1 min and the related sample at the given time. Values are means ± SE (nmoles/mg protein).
[b] Statistically different to respective control.

Table 2. Effect of chlorpromazine on initial velocity of efflux (Voe)[a] of taurocholate from hepatocytes

[TC][b]	Control	CPZ (100 μM)	P Value[c]
5	0.038 ± 0.002	0.035 ± 0.003	0.4651
10	0.071 ± 0.002	0.062 ± 0.006	0.1717
25	0.181 ± 0.007	0.151 ± 0.018	0.1305
50	0.341 ± 0.024	0.305 ± 0.013	0.0842
100	0.561 ± 0.052	0.556 ± 0.049	0.9802

[a] Voe in nmoles/min/mg protein calculated from the slopes of the lines for cell [TC] at 30, 60, 90, 120, 150 and 180 sec. Values are mean ± SE. N = 4.

[b] Taurocholate concentration (μM) used to pre-load hepatocytes.

[c] P value from paired *t*-test as determined for control vs chlorpromazine exposed Voe's.

sequently isolated hepatocytes showed no change in ability to take up taurocholate (Table 3). Initial experiments had shown that 48 hr pretreatment resulted in an increased uptake of taurocholate. In fact, if the current data for control vs 48 hr are analysed by *t*-test a significant difference is obtained. However, analysis of variance of all the times together does not provide evidence of statistical significance.

The effects of pretreatment of rats with chlorpromazine on taurocholate efflux are illustrated in Table 4 where the data are expressed as the difference for the respective value from the 1 min sample. It can be seen that none of the pretreatment schedules caused an alteration in the efflux of taurocholate.

Table 5 shows that taurocholate levels in the hepatocytes isolated from control and 48 hr pretreated rats are not different.

DISCUSSION

At concentrations of chlorpromazine from 1 to 100 μM in the incubation vessel no effect on uptake of the bile salt, sodium taurocholate, was found. However, at 50 and 100 μM chlorpromazine there was a clear inhibition of release of taurocholate from pre-loaded cells over an extended incubation period. Examination of the efflux process *per se*, however, showed that chlorpromazine (100 μM) had no significant effect on the initial velocity of this process (Voe) over a range of taurocholate pre-loading concentrations. Thus, as chlorpromazine clearly inhibits taurocholate release at 5–30 min but not the efflux process itself, it is possible that release of intracellular taurocholate could be inhibited in the presence of chlorpromazine. This could account for the lack of effect on the initial rate, which probably reflects readily available taurocholate, with a demonstrable effect at later sampling times, which probably also involves taurocholate bound in deeper intracellular compartments. The possibility for an effect on release only after 5 min of incubation being due to time required to attain appropriate intracellular levels of drug (or metabolite) was discounted as preincubation with chlorpromazine for 15 min in the pre-loading flask did not result in any evidence of inhibition of Voe (data not shown). Another study found that chlorpromazine (25 μM) was able to inhibit norepinephrine-induced taurocholate release from isolated hepatocytes, although the authors reported little or no perturbation in steady-state levels of intracellular taurocholate by chlorpromazine in the absence of the agonist [25].

After pretreating the rats with intraperitoneal chlorpromazine no effects on taurocholate transport in subsequently isolated hepatocytes were seen. In initial experiments there was a tendency observed to an increased uptake after treatment. This led to the rather extensive investigation of the several pretreatment times (2–60 hr) in order to attain confidence in the observations. Similarly with efflux, early experiments indicated some differences. Investigation of additional rats has led to the conclusion that the pretreatment does not result in an altered efflux of taurocholate from preloaded hepatocytes.

It is possible that the rate of exchange between endogenous and the exogenous radiolabelled taurocholate could be altered after chlorpromazine pretreatment and that this could confound inter-

Table 3. Effect of pretreatment of rats with chlorpromazine on uptake of taurocholate by subsequently isolated hepatocytes

Chlorpromazine[a] pretreatment (N)	Sample time (min)					
	1	2	5	10	20	30
0 (10)	1.68 ± 0.18[b]	3.03 ± 0.34	5.20 ± 0.39	6.47 ± 0.46	6.94 ± 0.45	7.08 ± 0.45
2 (7)	2.04 ± 0.16	3.60 ± 0.32	6.05 ± 0.38	7.00 ± 0.44	7.38 ± 0.46	7.42 ± 0.47
24 (7)	2.05 ± 0.21	3.54 ± 0.30	5.42 ± 0.35	7.07 ± 0.55	7.40 ± 0.57	7.21 ± 0.53
36 (3)	1.78 ± 0.20	3.52 ± 0.29	5.87 ± 0.36	7.08 ± 0.33	7.76 ± 0.39	7.82 ± 0.40
48 (8)	2.21 ± 0.25	3.95 ± 0.35	6.90 ± 0.59	8.17 ± 0.63	8.53 ± 0.67	8.31 ± 0.59
60 (3)	1.62 ± 0.05	3.20 ± 0.17	5.25 ± 0.27	6.41 ± 0.35	7.05 ± 0.33	7.24 ± 0.39

[a] Rats were pretreated with chlorpromazine (100 μmoles/kg) at the designated number of hours before liver perfusion for hepatocyte isolation. N = number of rats.

[b] Values are mean ± SE (nmoles/mg protein).

Table 4. Effects of pretreatment of rats with chlorpromazine on efflux of taurocholate from subsequently isolated hepatocytes

Chlorpromazine[a] pretreatment (N)	Sample time (min)				
	2	5	10	20	30
0 (7)	0.40 ± 0.05[b]	0.93 ± 0.08	1.21 ± 0.09	1.31 ± 0.11	1.34 ± 0.11
2 (4)	0.38 ± 0.02	0.91 ± 0.09	1.19 ± 0.17	1.19 ± 0.20	1.26 ± 0.20
24 (4)	0.36 ± 0.03	1.06 ± 0.07	1.46 ± 0.18	1.58 ± 0.28	1.65 ± 0.32
36 (3)	0.32 ± 0.02	0.97 ± 0.10	1.33 ± 0.16	1.42 ± 0.21	1.47 ± 0.24
48 (5)	0.30 ± 0.05	0.86 ± 0.09	1.11 ± 0.10	1.21 ± 0.13	1.23 ± 0.14
60 (3)	0.32 ± 0.06	0.93 ± 0.04	1.31 ± 0.04	1.49 ± 0.08	1.49 ± 0.07

[a] Rats were pretreated with chlorpromazine (100 μmoles/kg) at the designated number of hours before liver perfusion for hepatocyte isolation. N = number of rats.

[b] Values are mean ± SE (nmoles/mg protein). The values are the difference between a sample taken at 1 min and the related sample at the given time.

Table 5. Effect of pretreatment of rats with chlorpromazine on endogenous taurocholate in isolated hepatocytes

Chlorpromazine[a] pretreatment (N)	[TC][b]
0 (4)	40.3 ± 6.6[c]
48 (4)	31.2 ± 5.8

[a] Rats were pretreated with chlorpromazine (100 μmoles/kg) at the designated number of hours before hepatocyte isolation. N = number of rats.

[b] Taurocholate concentration (nmoles/g wet weight).

[c] Values are mean ± SE.

pretation of the data. Measurement of taurocholate in hepatocytes isolated 48 hr after chlorpromazine pretreatment did not reveal any differences to control rats, however. Other studies have reported data that allow approximation of taurocholate concentrations to 0.2–0.3 nmoles/mg protein in freshly isolated rat hepatocytes [26–28]. Our value of 40 nmoles/g cells is equivalent to this on conversion to a mg protein basis.

It can be seen from the data that if there is an inhibition by chlorpromazine of hepatocellular transport of taurocholate involved in biliary dysfunction it is not at the uptake step. Rather, there was a 50% reduction in release at 50 and 100 μM (Table 1). Thus, the data of this study do not support the contention of Tavoloni *et al.* [6] that chlorpromazine exerts its action on the biliary system by inhibiting bile salt uptake. It is noteworthy that the concentrations of the two studies are of the same order.

Concentrations of higher than 100 μM were not used in the current study to avoid cytotoxicity, which would obviously compromise a study of substrate transport. Under the experimental conditions, no cytotoxicity was observed. Others have reported a cytotoxic response of hepatocytes to chlorpromazine at 100 μM [29, 30]. However, both studies used cells at a lower concentration, which probably accounts for the difference in concentrations at which cytotoxicity is observed.

Blood levels of about 1 μM are generally accepted as therapeutic for chlorpromazine [31, 32]. An earlier study reported concentrations of about 10 μM [33], while it is thought that liver levels are about 10 times greater than this [34, 35]. The blood levels reported are not necessarily peak levels, however. Indeed, in a study of some 48 subjects, May *et al.* [36] found a wide range of peak plasma values for chlorpromazine. They indicated a spread of four to 20 times and found 10% of individuals may have values well beyond this and that unusual peak values may exceed the usual by as much as 100 times. One wonders if the extremes of peak plasma levels noted above are not related to the incidence of hepatotoxicity, which would support a direct mechanism of action. It should be appreciated that Ros *et al.* [5] reported biliary concentrations of chlorpromazine plus metabolites 74–1400-fold greater than serum concentrations, which also indicates a concentrating effect on passage through the hepatobiliary system. Even with these considerations, however, the extrapolation to the overt human response is tenuous. Perhaps the interference with the handling of taurocholate by chlorpromazine in rat hepatocytes is more relevant to the subclinical interference noted in a larger number of patients. It is likely that other factors would be involved in those individuals presenting with clear hepatotoxicity.

The actual mechanism by which chlorpromazine interferes with biliary function is still unclear. One possibility is the inhibition of NaK-ATPase [35] and this appears to be linked to taurocholate transport [37]. The data of the current experiments would not support this given that uptake is not inhibited by chlorpromazine and that NaK-ATPase has a non-canalicular distribution in the hepatocyte membrane [38]. A role for Mg-ATPase may also be doubtful given that efflux is apparently energy dependent [39] and this process was not inhibited by chlorpromazine. The role of alteration in membrane fluidity is perhaps also questioned given that NaK-ATPase activity seems to be correlated with this [40]. On the other hand, it is of interest to note that chlorpromazine causes an inhibition of uptake as well as secretion of horseradish peroxidase [8], which suggests that chlorpromazine may have other effects

on cellular transport of larger molecules. Alternatively, other of the multiple effects of chlorpromazine may be operative in this situation.

In conclusion, it has been shown that chlorpromazine at concentrations of up to 100 μM is unable to inhibit uptake of taurocholate by isolated hepatocytes. However, while efflux *per se* is not inhibited by direct addition of this drug, release of taurocholate over an extended incubation period is clearly decreased. Pretreatment of animals with chlorpromazine did not result in altered transport of the bile salt in subsequently isolated hepatocytes. The data do not support the contention that an inhibition of bile salt uptake at the sinusoid is responsible for the interference with biliary function by chlorpromazine but do suggest the possibility that hepatobiliary dysfunction may be related to altered intracellular handling of taurocholate.

Acknowledgements—The valuable technical assistance of Barbara Kotecka and the advice of John Mandryk on statistical analysis are gratefully acknowledged. Taurocholate assay by HPLC was carried out by John Earl, Biochemistry Department, The Children's Hospital, Camperdown, N.S.W.

REFERENCES

1. Plaa GL and Priestly BG, Intrahepatic cholestasis induced by drugs and chemicals. *Pharmacol Rev* **28**: 207–273, 1977.
2. Dossing M and Andreasen PB, Drug-induced liver disease in Denmark. *Scand J Gastroenterol* **17**: 205–211, 1982.
3. Elias E and Boyer JL, Mechanisms of intrahepatic cholestasis. In: *Progress in Liver Diseases* Vol. 6 (Eds. Popper H and Schaffner F), pp. 457–470. Grune & Stratton, New York, 1979.
4. Fisher MM, Mechanisms of drug-induced cholestasis. *Semin Liver Dis* **1**: 151–156, 1981.
5. Ros E, Small DM and Carey MC, Effects of chlorpromazine hydrochloride on bile salt synthesis, bile formation and biliary lipid secretion in the Rhesus monkey: a model for chlorpromazine-induced cholestasis. *Eur J Clin Invest* **9**: 29–41, 1979.
6. Tavolini N, Reed JS and Boyer JL, Effect of chlorpromazine on hepatic clearance and excretion of bile by the isolated perfused rat liver. *Proc Soc Exp Biol Med* **170**: 486–489, 1982.
7. Tsao SC, Sawada Y, Iga T and Hanano M, Effect of chlorpromazine on hepatic transport of Indocyanine Green in rats. *Biochem Pharmacol* **32**: 1105–1112, 1983.
8. Okanoue T, Kondo I, Ihrig TJ and French SW, Effect of ethanol and chlorpromazine on transhepatic transport and biliary secretion of horseradish peroxidase. *Hepatology* **4**: 253–260, 1984.
9. Simon RF and Reichen J, Bile secretory failure: recent concepts of the pathogenesis of intrahepatic cholestasis. In: *Progress in Liver Diseases*, Vol. 7 (Eds Popper H and Schaffner F), pp. 195–206. Grune & Stratton, New York, 1982.
10. Eaton DL and Klaassen CD, Effects of 2, 3, 7, 8-tetrachlorodibenzo-p-dioxin, Kepone, and polybrominated biphenyls on transport systems in isolated rat hepatocytes. *Toxicol Appl Pharmacol* **51**: 137–44, 1979.
11. Tarao K and Takamura Y, Bile acids excretion in isolated hepatocytes from cholestatic rat. *Jap J Gastroenterol* **77**: 744–749, 1980.
12. Tarao K, Olinger EJ, Ostrow JD and Balistreri WF, Impaired bile acid efflux from hepatocytes isolated from the liver of rats with cholestasis. *Am J Physiol* **243**: G253–G258, 1982.
13. Yang KH, Choi EJ and Choe SY, Cytotoxicity of 2, 3, 7, 8, tetrachlorodibenzo-p-dioxin on primary culture of adult rat hepatocytes. *Arch Environ Contam Toxicol* **12**: 183–188, 1983.
14. Berr F, Simon FR and Reichen J, Ethynylestradiol impairs bile salt uptake and Na-K pump function of rat hepatocytes. *Am J Physiol* **247**: G437–G443, 1984.
15. Brown DJ and Hunter A, The effect of thioacetamide on sulfobromophthalein and ouabain transport in isolated rat hepatocytes. *Toxicology* **31**: 165–176, 1985.
16. Schwarz LR, Schwenk M, Pfaff E and Greim H, Cholestatic steroid hormones inhibit taurocholate uptake into isolated rat hepatocytes. *Biochem Pharmacol* **26**: 2433–2437, 1977.
17. Cordiner SJ and Jordan TW, Inhibition by sporidesmin of hepatocyte bile acid transport. *Biochem J* **212**: 197–204, 1983.
18. Stacey NH, Effects of ethinyl estradiol on substrate uptake and efflux by isolated rat hepatocyte. *Biochem Pharmacol* **35**: 2495–2500, 1986.
19. Berry MN and Friend DS, High yield preparation of isolated rat liver parenchymal cells; a biochemical and fine structural study. *J Cell Biol* **43**: 506–520, 1969.
20. Stacey NH, Cantilena LR Jr. and Klaassen CD, Cadmium toxicity and lipid peroxidation in isolated rat hepatocytes. *Toxicol Appl Pharmacol* **53**: 470–480, 1980.
21. Stacey NH and Klaassen CD, Cadmium uptake by isolated rat hepatocytes. *Toxicol Appl Pharmacol* **55**: 448–455, 1980.
22. Lowry OH, Rosebrough NJ, Farr AL and Randall RJ, Protein measurement with the Folin phenol reagent. *J Biol Chem* **193**: 265–275, 1951.
23. Eaton DL and Klaassen CD, Carrier-mediated transport of ouabain in isolated hepatocytes. *J Pharmacol Exp Ther* **205**: 480–488, 1978.
24. Nakayama F and Nakagaki M, Quantitative determination of bile acids in bile with reversed-phase high-performance liquid chromatography. *J Chromatogr* **183**: 287–293, 1980.
25. Gewirtz DA, Randolph JK and Goldman ID, Induction of taurocholate release from isolated rat hepatocytes in suspension by adrenergic agents and vasopressin: implications for control of bile salt secretion. *Hepatology* **4**: 205–212, 1984.
26. Yousef IM, Ho J and Jeejeebhoy KN, Bile acid synthesis in isolated rat hepatocytes. *Can J Biochem* **56**: 780–783, 1978.
27. Whiting MJ and Edwards AM, Measurement of cholic acid synthesis and secretion by isolated rat hepatocytes. *J Lipid Res* **20**: 914–918, 1979.
28. Hashimoto S, Uchida K and Hirata M, Hepatic bile acid elution by albumin and bile acid content in isolated rat hepatocytes. *Lipids* **17**: 149–154, 1982.
29. Tsao SC, Iga T, Sugiyama Y and Hanano M, Effect of chlorpromazine on isolated rat hepatocytes. *Biochem Pharmacol* **31**: 491–497, 1982.
30. Utili R, Abernathy CO, Zimmerman HJ, Gaeta GB, Adinolfi L and Lukacs L, Endotoxin protects against chlorpromazine-induced cholestasis in the isolated perfused rat liver. *Gastroenterology* **80**: 673–680, 1981.
31. Cooper TB, Plasma level monitoring of antipsychotic drugs. *Clin Pharmacokinetics* **3**: 14–38, 1978.
32. Benet LZ and Sheiner LB, Appendix II. Design and optimization of dosage regimens; pharmacokinetic data. In: *The Pharmacological Basis of Therapeutics* (Eds. Gilman AG, Goodman LS and Gilman A), p. 1696. Macmillan, New York 1980.
33. Huang C and Kurland AA, Chlorpromazine blood levels in psychotic patients. *Arch Gen Psychiatry* **5**: 509–513, 1961.

34. Tavoloni N, Reed JS, Hruban Z and Boyer JL, Effect of chlorpromazine on hepatic perfusion and bile secretory function in the isolated perfused rat liver. *J Lab Clin Med* **94**: 726–741, 1979.
35. Keefe EB, Blankenship NM and Scharschmidt BF, Alteration of rat liver plasma membrane fluidity and ATPase activity by chlorpromazine hydrochloride and its metabolites. *Gastroenterology* **79**: 222–231, 1980.
36. May PRA, Van Putten T, Jenden DJ, Yale C and Dixon WJ, Chlorpromazine levels and the outcome of treatment in schizophrenic patients. *Arch Gen Psychiatry* **38**: 202–207, 1981.
37. Boyer JL, New concepts of mechanisms of hepatocyte bile formation. *Physiol Rev* **60**: 303–326, 1980.
38. Blitzer BL and Boyer JL, Cytochemical localization of Na^+, K^+-ATPase in the rat hepatocyte. *J Clin Invest* **62**: 1104–1108, 1978.
39. Schwarz LR, Schwenk M, Pfaff E and Greim H, Excretion of taurocholate from isolated hepatocytes. *Eur J Biochem* **71**: 369–373, 1976.
40. Schachter D, Fluidity and function of hepatocyte plasma membranes. *Hepatology* **4**: 140–151, 1984.