in pentobarbital excretion were seen in both the 10 mg/hr and 30 mg/hr taurocholate-perfused groups.

Taurocholate perfusion had no significant effect on the form in which pentobarbital was excreted into bile (Table 2). Less than 2 per cent of total biliary radioactivity remained as unmetabolized pentobarbital. Metabolites extracted into ethyl acetate and those metabolites remaining in the bile-buffer mixture after ethyl acetate extraction did not move from the origin when chromatographed on Silica gel G in chloroform–acetone (9:1, v/v). Further identification of pentobarbital metabolites was not attempted.

Biliary excretion of pentobarbital metabolites was increased in the rat by bile salt perfusion. Interdependence between bile flow rates and biliary excretion could explain the increased pentobarbital excretion in response to taurocholate perfusion. Such interdependence has been shown for digitoxin [12], propylthiouracil [13], and cholecystography contrast agents [14]. In addition, hypothermia produced pronounced decreases in bile flow and markedly reduced excretion of pentobarbital into bile in the isolated perfused rat liver [15]. Direct interaction between pentobarbital metabolites and taurocholate is a second possibility. Physicochemical interaction between BSP and taurocholate to form a large macromolecular complex has been demonstrated *in vitro* [16], and it has been postulated that the increased T_m for BSP during bile salt infusion represents a specific effect of the bile salt rather than being attributable to bile salt-induced choleresis. It is possible that taurocholate combines with pentobarbital metabolites to form a complex which is then secreted into the biliary canaliculus. Whether enhanced biliary excretion of active pentobarbital metabolites by bile salt-induced choleresis actually decreases pentobarbital toxicity remains to be determined. Accumulation of a primidone metabolite has been shown to reduce primidone metabolism in the isolated perfused rat liver [17], so depletion of intrahepatic pools of metabolites may play some role in facilitating metabolism of parent compounds. Numerous other potentially toxic compounds such as digitoxin and chlorpromazine are excreted in bile as the unchanged parent compound or as pharmacologically active metabolites [12, 18]. If bile salt-induced choleresis can be shown to increase hepatic drug metabolism by depletion of intrahepatic metabolites or to enhance biliary excretion of unchanged parent compounds or active metabolites, it may be a useful means of rapidly manipulating hepatic excretory function.

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Department of Hematology,
Walter Reed Army Institute of Research,
Walter Reed Army Medical Center,
Washington, D. C. 20012, U.S.A.

ROBERT G. KNODELL†
EDWARD E. HOLLOWAY

† Reprint requests should be sent to RGK, Division of Gastroenterology, University of New Mexico School of Medicine, Albuquerque, N.M., 87131.

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Comparison of the inhibition of 5-hydroxytryptamine uptake by methadone and its congeners in human platelets

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Recent studies have indicated that methadone is a potent inhibitor of 5-hydroxytryptamine (5-HT) uptake by rabbit brain synaptosomes with a K_i of approximately 10^{-9} M [1]. Despite this low K_i, no depletion of brain 5-HT during treatment of animals with methadone has been reported [2, 3]. Apparently, the rate of endogenous synthesis of 5-HT in the brain is sufficient to maintain the normal levels.

In contrast to 5-HT-containing neurons in the brain, it is thought that platelets derive most of their 5-HT stores from exogenous sources [4]. Consequently, it is possible to deplete platelet 5-HT in man by uptake blockers, such as imipramine [5]. Although methadone has also been found to inhibit the uptake of 5-HT by human platelets *in vitro*, the concentration of methadone reported to cause a 50 per cent inhibition of uptake (2.1×10^{-5} M) [6] appears to be much greater than the concentration required to inhibit uptake by synaptosomes [1]. The relatively large concentration of methadone reportedly required to inhibit human platelet 5-HT uptake [6] would

appear to indicate little or no effect of methadone maintenance on platelet 5-HT content *in vivo*, because the concentration of 2.1×10^{-5} M is about one order of magnitude greater than the concentration of methadone in the plasma of patients maintained on a daily oral dose of 100–120 mg [7]. The studies *in vitro* with human platelets, however, were carried out with excessively large concentrations of 5-HT or relatively long incubation times (10^{-5} M for 15 min or 10^{-6} M for 1 hr) [6]. Tuomisto [8] has demonstrated that long incubation times or large concentrations of 5-HT can lead to falsely low potencies for uptake inhibitors, due to nonlinearity of the rate of uptake or to diffusion respectively.

The purpose of the present study was to determine whether methadone caused an appreciable inhibition of uptake of 5-HT by human platelets at concentrations that were likely to be encountered in a methadone-maintenance situation. We also studied the effects of several other methadone congeners in an attempt to identify a structural requirement for uptake inhibition. Imipramine and chlorimipramine were also tested for comparison, because these are known to be very potent inhibitors of 5-HT uptake [5, 6, 9].

Fresh human platelet-rich plasma was obtained from the Irwin Memorial Blood Bank of the San Francisco Medical Society. The standard incubation mixture contained 0.5 ml of platelet-rich plasma and 1.5 ml of a modified calcium-free Tyrode's solution described previously [6]. The incubations were carried out for 4 or 5 min in plastic scintillation vials using a Dubnoff metabolic shaking incubator at 37°. The incubation mixture contained 0.92×10^{-7} M of 1,2-[^{3}H]5-HT (sp. act.: 2.72 mCi/μmole; New England Nuclear Corp.) and 5×10^{-7} M of unlabeled 5-HT (Sigma Chemical Co.). The incubation mixture was preincubated at 37° for 5 min before the addition of platelet-rich plasma. All incubations were carried out in duplicate. The incubation was stopped by the addition of 6 ml of chilled Tyrode's solution. The platelets were collected by vacuum filtration on Whatman GF/C glass fiber discs with a diameter of 2.4 cm and were washed twice with 8-ml aliquots of chilled Tyrode's solution. The discs were dried and counted in 7.5 ml ScintiVerse (Fisher Scientific Co.) using a Packard Tri-Carb liquid scintillation spectrometer.

The following drugs and methadone metabolites were gifts from the Lilly Laboratories, Eli Lilly & Co.: (+) and (−) methadone hydrochloride; (+) and (−) α-methadol hydrochloride; (+) and (−) β-methadol; (+) and (−) α-acetyl-methadol hydrochloride; (+) and (−) β-acetyl-methadol hydrochloride; (−) α-nor-methadol hydrochloride; (±) 1,5-dimethyl-3,3-diphenyl-2-ethylidene pyrrolidine perchlorate (referred to in this paper as metabolite A); and 3,3-diphenyl-2-ethyl-5-methyl-1-pyrrolidine hydrochloride (referred to in this paper as metabolite B). (±) Methadone hydrochloride was obtained from Mallinckrodt. Imipramine hydrochloride and chlorimipramine hydrochloride were gifts from CIBA-Geigy. The drugs were dissolved in the modified Tyrode's solution. The concentration of drug causing 50 per cent inhibition of uptake (IC_{50}) in the presence of 5.9×10^{-7} M 5-HT was determined graphically by log-probit analysis [10], using three or four concentrations of the drug.

The K_m for 5-HT uptake was $3.81 \pm 0.15 \times 10^{-7}$ M (mean ± S. D. for three experiments), which is slightly lower than the K_m of approximately 6×10^{-7} M reported by Lingjaerde [11]. The maximum velocity of 5-HT uptake in the absence of inhibitors varied with different preparations of platelet-rich plasma and ranged from 37.2 to 99.5 pmoles/ml of plasma/min. The uptake of 5-HT at a concentration of 5.9×10^{-7} M was inhibited over 99 per cent by 3×10^{-6} M chlorimipramine, 9×10^{-6} M imipramine, 3×10^{-5} M (−) methadone and 3×10^{-4} M (+) α-methadol. This indicated that very little of the uptake at this concentration of 5-HT was due to simple diffusion.

Figure 1 shows that the inhibition of 5-HT uptake by (−) methadone appears to be competitive. The apparent K_i for (−) methadone was $7.6 \pm 1.7 \times 10^{-8}$ M (mean ± S. D. for three experiments).

As shown in Table 1, all of the drugs and metabolites tested inhibited the uptake of 5-HT. Chlorimipramine, the most potent drug tested, was ten times as potent as (−) methadone. Although 5-HT does not possess an asymmetric carbon atom, the uptake inhibitors displayed stereoselectivity. For example, (−) methadone was 30 times as potent as (+) methadone, and (+) α-methadol was about 26 times as potent as (−) α-methadol. In general, the potency for 5-HT uptake inhibition did not parallel the analgesic potency determined in mice by Eddy *et al.* [13], but within certain groups (methadone and acetylmethadol) some enantiomorphic parallelism between uptake inhibition and analgesia was observed. The potency for 5-HT uptake inhibition within the methadol group also did not parallel the potency for inhibition of tritiated dihydromorphine (DHM) binding to the synaptic plasma membrane fraction from rat brain, as determined by Terenius [14]. These findings are in agreement with a previous report that the inhibition of platelet 5-HT uptake by narcotic drugs does not parallel their analgesic potency [6]. Thus, the uptake carrier for 5-HT in platelet membranes does not appear to be a suitable model for the opiate receptor.

There was a more striking parallelism, however, between uptake inhibition by methadone and its congeners and the absolute configuration of the enantiomorphs which possessed an asymmetric center in common with methadone. Thus, the compounds with the 6R configuration, such as (−) methadone, (+) α-methadol, (+) α-acetylmethadol, (−) β-methadol and (−) β-acetylmethadol, were more potent uptake inhibitors than the compounds with the 6S configuration, such as (+) methadone, (−) α-methadol, (−) α-acetylmethadol, (+) β-methadol, and (+) β-acetylmethadol.

The results above indicate the possibility that platelets from patients in a methadone-maintenance program may have a decreased ability to take up 5-HT *in vivo*. The extent of this decrease will depend on primarily upon the concentrations of unbound (−) methadone and 5-HT in the plasma. The extent of inhibition shown in the present study may be greater than that occurring *in vivo*, due to the fact that about 85 per cent of the (−) methadone in plasma is protein bound [15]. Since the incubations in the present study contained only 25 per cent plasma, a greater proportion of methadone in the incubations may be expected to be unbound [16], when compared to whole

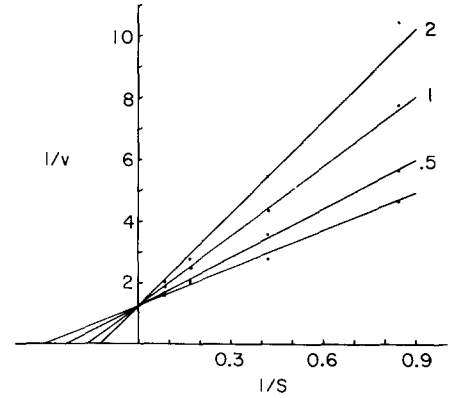

Fig. 1. Double reciprocal plots of 5-HT uptake in the presence of (—) methadone at concentrations of 0, 0.5, 1.0 and 2.0×10^{-7} M. The incubations were carried out for 4 min. The 5-HT concentrations ranged from 1.18 to 11.8×10^{-7} M. Velocity is expressed as moles $\times 10^{-11}$/ml of plasma/min.

Table 1. Inhibition of 5-HT uptake by chlorimipramine, imipramine, methadone and its congeners

Compound	Configuration*	IC_{50} (M) for 5-HT uptake†	ED_{50} for analgesia‡	IC_{50} (M) for DHM binding§	Slope ± S. E.
Chlorimipramine		1.1 ± 0.43 (10^{-8})			0.689 ± 0.141
Imipramine		2.8 ± 0.82 (10^{-8})			0.616 ± 0.053
(−) Methadone	6R	1.1 ± 0.15 (10^{-7})	0.8	4 (10^{-9})	0.549 ± 0.034
(±) Methadone		2.5 ± 0.35 (10^{-7})	1.6		0.659 ± 0.024‖
(+) Methadone	6S	3.3 ± 0.81 (10^{-6})	25.7	1 (10^{-7})	0.841 ± 0.079‖
(+) α-Methadol	3R:6R	6.2 ± 0.93 (10^{-7})	24.7	1 (10^{-6})	0.640 ± 0.044
(−) β-Methadol	3S:6R	1.8, 1.7 (10^{-6})	7.6	3 (10^{-8})	0.681 ± 0.024‖
(−) α-Methadol	3S:6S	1.6 ± 0.62 (10^{-5})	3.5	7 (10^{-8})	0.631 ± 0.086
(+) β-Methadol	3R:6S	2.1, 2.4 (10^{-5})	63.7	1 (10^{-5})	0.670 ± 0.332
(+) α-Acetylmethadol	3R:6R	1.2 ± 0.06 (10^{-6})	0.3		0.745 ± 0.024‖
(−) β-Acetylmethadol	3S:6R	2.3 ± 0.30 (10^{-6})	0.4		0.787 ± 0.030‖
(−) α-Acetylmethadol	3S:6S	9.7 ± 1.4 (10^{-6})	1.8		0.721 ± 0.048‖
(+) β-Acetylmethadol	3R:6S	3.3 ± 0.50 (10^{-5})	4.1		0.717 ± 0.119
Metabolite A		1.2 ± 0.00 (10^{-6})			0.635 ± 0.017
(−) α-Normethadol	(3S:6S)	7.9 ± 0.92 (10^{-6})			0.605 ± 0.034
Metabolite B		9.7 ± 2.5 (10^{-4})			0.661 ± 0.075

* From Ref. 12.

† Incubations were carried out for 5 min at a 5-HT concentration of 5.9×10^{-7} M. Data are expressed as the mean ± S. D. for three experiments, with the exception of (+) and (−) β-methadol (two experiments).

‡ Data from Ref. 13 showing s.c. ED_{50} values (mg/kg) in mice, using the hot plate method.

§ Data from Ref. 14 showing IC_{50} values for inhibition of tritiated dihydromorphine (DHM) binding to the synaptic plasma membrane fraction of rat brain.

‖ Significantly different from the slope for (−) methadone, P = 0.05 or less.

plasma. However, since a significant amount of protein binding of (−) methadone would be expected to occur in the incubations [16], the actual K_i for (−) methadone inhibition of 5-HT uptake by platelets may be less than the apparent K_i of 7.6×10^{-8} M that was observed in the presence of 25 per cent plasma.

Imipramine, which in the present study was about four times as potent as (−) methadone, has been reported to decrease human platelet 5-HT to 17 per cent of the control levels after 3 weeks of a daily oral dose of 150–300 mg [5]. Part of this depleting action may be due to the formation of desmethylimipramine, which also depletes platelet 5-HT [17]. It is not known at the present time whether a depletion of platelet 5-HT occurs during methadone maintenance.

In summary, the two significant findings of the present study are: first, that the inhibition of platelet 5-HT uptake by methadone and its congeners shows structural specificity in that compounds with the 6R configuration were more potent than compounds with the 6S configuration; and, second, that methadone is a more potent inhibitor of 5-HT uptake by platelets than was previously recognized, which raises the possibility that methadone maintenance may cause depletion of platelet 5-HT *in vivo*.

DAVID A. BRASE
HORACE H. LOH
Department of Pharmacology and Langley Porter Neuropsychiatric Institute, University of California at San Francisco, San Francisco, Calif. 94143, U.S.A.

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