production nor altered the increase in cyclic AMP caused by histamine or LSD (results not shown). These results are in agreement with the hypothesis that the increase in cyclic AMP and AP uptake caused by LSD is the consequence of LSD interacting with histamine receptors.

Since in our preparation the actions of various histamine agonists or antagonists with histamine receptors were shown to be similar to their actions on H_2-receptors in other tissues [1, 2, 6], and because of the close correlation between histamine binding and the biological response [11], we concluded that LSD interacts with histamine H_2-receptors located on the parietal cells.

Our present findings are similar to those of others [3, 4, 8], who reported that histamine receptors interact with LSD. In the brian tissue, LSD acted as a competitive antagonist of histamine [3, 4], whereas in guinea pig right atrium it acted as a partial agonist [8]. In our preparation, the affinity for LSD was reasonably close to that reported for the brian or atrium, and LSD acted as a partial agonist. Recent reports also revealed that both H_1- and H_2-antagonists inhibited competitively the histamine-activated adenylate cyclase in membrane preparations from heart [5] and brain tissues [3]. The relative potencies of histamine antagonists in those preparations were close to their relative potencies in our preparation [6], and the potencies of the H_2-antagonists were similar to their potencies on other functions which are considered to be mediated by H_2-receptors (e.g. acid secretion and contraction of uterine or atrial smooth muscle) [1, 2]. These observations suggest that the action of histamine on gastric cells as well as on heart and brain tissues is mediated by H_2-receptors. The later tissues appear to be different from other tissues (rat uterus, guinea pig atrium) in their responses to H_1-antagonists. The H_2-receptor-mediated responses of rat stomach (acid secretion) and guinea pig atrium (smooth muscle contraction) were not shown to be inhibited by H_1-antagonists [2, 7]. In contrast, in gastric cells, heart and brain tissues, H_1-antagonists are relatively high concentrations inhibited the tissue response to histamine. Whether this inhibition is of the *fully* competitive type (i.e. H_1- and H_2-antagonists interact with the histamine receptors) or of the *partially* competitive type [6] (H_1-antagonists interact with a site, distinct from the histamine receptor to reduce the affinity for histamine) remains to be determined.

In summary in dispersed mucosal cells from guinea pig stomach, D-lysergic acid diethylamide (LSD) was a partial agonist with respect to histamine. LSD, like histamine, inhibited [^{3}H]histamine binding and increased both cellular cyclic AMP and [^{14}C]aminopyrine uptake by interacting with histamine H_2-receptors on parietal cells. These processes were blocked by both histamine H_1- and H_2-antagonists and, thus, provide evidence that histamine H_2-receptors on guinea pig parietal cells resemble those in brain tissue in that they interact with LSD as well as with both classes of histamine antagonists.

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Inhibitory effect of tranylcypromine on hepatic drug metabolism in the rat

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Tranylcypromine [*trans*-(±)-2-phenylcyclopropylamine], an inhibitor (MAOI) of monoamine oxidase [amine:oxygen oxidoreductase (deaminating) flavin containing; EC 1.4.3.4], is used in therapeutics as an antidepressive agent [1]. Like many other MAOI, its clinical use is impaired by its potential toxicity and its involvement in many drug interactions of which those with MAOI and hypnotics, narcotic analgesics and tricyclic antidepressants are of clinical importance [2–4]. The mechanisms of many of these drug interactions have not been elucidated, but it has been proposed that MAOI depression of the hepatic metabolizing enzyme systems responsible for the elimina-

tion of these therapeutic agents is the basis of these interactions [2, 3]. It has been reported that iproniazid [5–8], nialamide [6] and phenelzine [2, 9] inhibit the *in vitro* microsomal metabolism of drugs. A more recent study established that pretreatment of rats which doses of 75 mg/kg of pargyline depresses the *N*-demethylation of ethylmorphine [10]. The effect of tranylcypromine on hepatic drug metabolism has not been reported.

Recently, we reported a comparative study of the inhibitory effects of iproniazid, nialamide, pargyline, phenelzine and tranylcypromine, all MAOI, on microsomal metabolism in rat liver [11]. It was found that the addition of 1 mM of any one of the above MAOI to the microsomal incubation mixtures decreased significantly oxidative reactions, such as *N*- and *O*-dealkylation, and aromatic hydroxylation, and that tranylcypromine had the most depressive effect. None of these MAOI had any effect on other microsomal reactions such as those of procaine hydrolase, *p*-nitrobenzoic acid reductase, NADPH–oxidase and NADPH–cytochrome *c* (P-450) reductase. The characteristics of the inhibitory effect of tranylcypromine on microsomal oxidative metabolism using rat liver preparations are now reported.

Materials and methods

Materials and animals. All the chemicals used were analytical grade and were obtained from the usual commercial sources. Tranylcypromine hydrochloride, nicotinamide, NADP, glucose-6-phosphate, 2-methyl-1,2-di-3-pyridyl-1-propanol (metyrapone), *n*-octylamine and serum bovine albumin fraction V were purchased from the Sigma Chemical Co., St. Louis (MO). Male, albino, Sprague–Dawley rats weighing 200–300 g at the time of experimentation were purchased from Canadian Breeding Farm and Laboratories Ltd., St-Constant, Québec, Canada. The animals were acclimatized to the animal house under an alternating 12 hr light/12 hr dark cycle for at least 1 week before the experiment. Spectrophotometric studies were carried out using a Unicam SP-800A spectrophotometer equipped with an expansion scale factor connected to a Hewlett–Packard X-Y recorder model 7044A.

Liver microsomal preparations. Rats were decapitated after an overnight fast, and the livers were immediately removed, weighed, cut into small pieces and homogenized in Tris–KCl buffer, pH 7.4, to give a suspension equivalent to 250 mg/ml of wet liver. Preparations of 10,000 *g* supernatant fraction and microsomes were carried out as described elsewhere [12]. The homogenate preparations used for the *p*-aniline hydroxylase assays were prepared in Tris–KCl buffer containing 1 mM EDTA. The concentration of cytochrome P-450 in the microsomal suspension was measured from the difference in absorbance of the reduced carbon monoxide–cytochrome P-450 complex between 450 and 500 nm according to Omura and Sato [13]. The protein content of the microsomal pellets was measured according to Lowry *et al.* [14] with serum bovine albumin as the standard.

Spectral interactions with oxidized cytochrome P-450. The interaction of the MAOI with oxidized cytochrome P-450 was determined by differential spectrometry using the microsomal suspensions at protein concentrations of 1, 1.4 and 2 mg/ml. Spectral changes and the apparent dissociation constant (K_s) were evaluated as described by Schenkman *et al.* [15].

Enzyme assays. All assays were carried out on the same day the tissue was prepared. Determinations of aminopyrine and *N,N*-dimethylaniline *N*-demethylase and *p*-nitroanisole *O*-demethylase were carried out with the assay for formaldehyde using the Nash reaction as described by Mazel [16]. Aniline hydroxylase was assayed as reported by Mazel [17] by measuring the amount of *p*-aminophenol formed with the indophenol reaction according to the method of Imai *et al.* [18]. It has been reported that, due to the presence of the soluble fraction, the recovery of the *p*-aminophenol formed in the aniline hydroxylase assay according to the method of Imai *et al.* is decreased by 10–25% depending upon the concentration of the metabolite [19]. To compensate for this loss, calibration curves of *p*-aminophenol were therefore constructed by adding different amounts of *p*-aminophenol (10–75 nmoles) in the proper dilution of soluble fraction. The above enzyme assays were carried out under linear kinetic conditions using the 10,000 *g* supernatant fraction as the microsomal preparation. The concentrations of the various substrates used in the incubation mixtures were 1, 2, 3 and 5 mM and those of tranylcypromine were 0.05, 0.1 and 0.3 mM. The MAOI was added to the incubate in phsophate buffer prior to the substrate solution.

Results

Inhibition studies. The microsomal reactions determined were the *N*-demethylation of aminopyrine and *N,N*-dimethylaniline, the *O*-demethylation of *p*-nitroanisole, and the ring hydroxylation of aniline. These oxidative reactions are mediated by the cytochrome P-450 mixed function oxidase system. In the case of *N,N*-dimethylaniline, it has been reported that this substrate can also be metabolized by *N*-oxidation and by *N*-demethylation by a flavin-dependent amine oxidase present in hepatic microsomal preparations [20, 21]. The latter enzyme system is differentiated from the cytochrome P-450 catalyzed reaction by its resistance to the inhibition of metyrapone and its activation by the addition of *n*-octylamine to the incubation mixtures [22]. Thus, the effect of the addition of 1 mM concentrations of metyrapone and *n*-octylamine on the *N*-demethylation of an equimolar amount of *N,N*-dimethylaniline was studied in order to examine the contribution of the flavine-dependent amine oxidase which might participate in the dealkylation of the substrate along with the cytochrome P-450 system. The concentrations of substrate, *n*-octylamine and metyrapone are those used by Ziegler *et al.* [22]. The incubations were carried out in triplicate at 37° for 20 min using the 10,000 *g* supernatant fraction of rat liver, and the enzymic activities are expressed as nmoles of formaldehyde formed per mg of microsomal protein per 10 min. A mean activity value of 87.2 nmoles per mg per 20 min (S.E. = 3.1) was obtained after the incubation of *N,N*-dimethylaniline with the microsomal fraction. The addition of metyrapone and *n*-octylamine to the incubation mixture decreased significantly the demethylase activity to mean values of 54.9 (S.E. = 2.5) and 27.4 (S.E. = 1.5) nmoles per mg per 20 min respectively. These results suggest that *N,N*-dimethylaniline is not demethylated by the flavine-dependent amine oxidase under the present conditions of assay.

Table 1 summarizes the inhibitory effects of tranylcypromine on the oxidative microsomal reactions of rat liver. Enzymic activities are expressed as nmoles of metabolites formed per mg of microsomal protein per time of incubation. Each concentration of substrate was incubated in triplicate with or without tranylcypromine. The data were plotted according to the Lineweaver–Burk method to give the apparent K_m and V_{max} values. Due to unspecific binding of the substrate to the soluble fraction present in the 10,000 *g* supernatant fraction, the apparent K_m values determined are higher than those reported in isolated microsomes, although the maximum rates of these reactions are in the same range of values as those obtained using microsomal pellets as the enzyme source [23]. Apparent K_i values were determined using the method described by Dixon [24]. Intercepts, slopes and points of intersection were calculated from linear regressions of each experiment. Under these conditions, the correlation coefficient of the regression lines was always higher than 0.90 in the concentration range of substrate and tranylcypromine used. Preliminary experiments in this laboratory showed that tranylcypromine was biotransformed when incubated with microsomal homogenates of rat liver under similar condi-

Table 1. Effects of preincubation of tranylcypromine with microsomal homogenate for 30 min at 37° on the inhibition of oxidative microsomal reactions of rat liver

Reactions	Concentration of tranylcypromine (mM)	Inhibition type		App. K_m (mM)		V_{max}* (nmoles/mg/20 or 30 min)		App. K_i (mM)	
		No Pre.†	Pre.†	No Pre.	Pre.	No Pre.	Pre.	No. Pre.	Pre.
Aminopyrine *N*-demethylase	0.0	C‡	C	0.9	0.7	133.3	90.9	0.07	0.11
	0.05			2.5		133.3			
	0.10			4.0	2.0	133.3	90.9		
	0.30			5.7	4.0	133.3	90.9		
N,N-Dimethylaniline *N*-demethylase	0.0	NC§	NC	1.9	1.5	166.6	142.8	0.15	0.30
	0.05			1.9	1.5	142.7	117.3		
	0.10			1.9	1.5	105.3	99.5		
	0.30			1.9	1.5	66.7	71.4		
p-Nitroanisole *O*-demethylase	0.0	C	C	0.6	0.5	51.6	55.2	0.025	0.050
	0.05			2.9	2.0	51.6	55.2		
	0.10			5.0	2.5	51.6	55.2		
	0.30			16.7	5.0	51.6	55.2		
Aniline hydroxylase	0.0	NC	NC	0.8	0.7	28.6	25.0	0.10	0.19
	0.05			0.8	0.7	11.8	16.9		
	0.10			0.8	0.7	9.1	13.5		
	0.30			0.8	0.7	5.4	7.4		

* Values are in nmoles of formaldehyde formed per mg protein per 20 min for the *N*-dealkylation reactions and nmoles of *p*-aminophenol formed per mg protein per 30 min for the aniline hydroxylase.

† Preincubation.

‡ C = competitive.

§ NC = non-competitive.

tions (P. M. Bélanger, unpublished results). Therefore, the effects of 30 min of preincubation with tranylcypromine (of the microsomal homogenate preparation in the presence of cofactor) prior to substrate addition were also studied. Tranylcypromine inhibited competitively the oxidative dealkylation of aminopyrine and *p*-nitroanisole and non-competitively the *N*-demethylation of *N,N*-dimethylaniline and aniline hydroxylase as shown by its effect on the apparent K_m and V_{max} values of these substrates in the absence and presence of the MAOI. The addition of various concentrations of tranylcypromine to the incubation mixtures of aminopyrine and *p*-nitroanisole resulted in corresponding increases in their apparent K_m values but did not modify their V_{max} values. Conversely, the V_{max} values of the *N,N*-dimethylaniline *N*-demethylase and aniline hydroxylase decreased with the addition of increasing amounts of tranylcypromine, while the apparent K_m values of these substrates were not affected by tranylcypromine. The preincubation for 30 min at 37° with or without tranylcypromine resulted in a general decrease in the apparent K_m values of all the substrates studied and of their V_{max} values, with the exception of the *O*-demethylation of *p*-nitroanisole. Preincubation with tranylcypromine prior to substrate addition did not modify the qualitative aspect of its inhibitory characteristics. Quantitatively, preincubation with tranylcypromine resulted in a significant reduction in its inhibitory effect. This is best estimated by the effect of the preincubation period on the apparent inhibitory constant (K_i) of tranylcypromine. The K_i values of tranylcypromine roughly doubled after the preincubation period, i.e. tranylcypromine had half the inhibitory capacity after preincubation for 30 min when compared to the values obtained when the MAOI and the substrate were added at the same time. In this case, the apparent K_i values calculated referred to the initial concentration of tranylcypromine added before the preincubation.

Binding studies. Figure 1 illustrates the spectral changes obtained by the addition of various concentrations of tranylcypromine to oxidized microsomes of rat liver. The peak variations in absorbance values were obtained at 430 nm and the trough values were located in the region of 392–406 nm. This spectrum is typical of a type II compound as described by Schenkman *et al.* [15]. The apparent dissociation constant (K_s) of 0.3 mM was calculated from the intercept on the abscissa of the double-reciprocal plot of the variation in absorbance between the peak and the trough values versus the concentration of tranylcypromine obtained at different microsomal protein concentrations. This constant represents the concentration of tranylcypromine that produces half the maximum spectral change and consequently is indicative of the capacity of tranylcypromine to bind the microsomal hemoprotein cytochrome P-450 [15, 25].

Discussion

The results obtained show that tranylcypromine is a powerful inhibitor of oxidative microsomal reactions of rat liver. Indeed, the addition of 0.05 to 0.3 mM tranylcypromine in the incubation mixtures containing 1–5 mM aminopyrine, aniline, *p*-nitroanisole or *N,N*-dimethylaniline resulted in significant inhibition of the biotransformation of these substrates. We reported recently that the depressive effect of MAOI on hepatic microsomal reactions was specific to the oxidative biotransformations of xenobiotics [11] and that these agents had no effect on other microsomal reactions of rat liver such as those of procaine hydrolase, *p*-nitrobenzoic acid reductase, NADPH-oxidase and NADPH-cytochrome *c* (P-450) reductase. Thus, the inhibitory effect of tranylcypromine cannot be explained by an increased rate of NADPH oxidation or a decrease in the reduction rate of the microsomal electron carrier cytochrome *c*.

The type of inhibition caused by tranylcypromine varied depending on the substrate used. Tranylcypromine inhibited competitively the aminopyrine and *p*-nitroanisole demethylases, but its depressive effects on the demethylation of *N,N*-dimethylaniline and on the aromatic hydroxylation of aniline were of a non-competitive type. The inhibitory effect of the MAOI was of the same order of intensity regardless of the substrate incubated.

This disparity of effect may be the result of different types of interaction with cytochrome P-450, the terminal oxidase involved in these reactions [26–28]. Aminopyrine has been reported to induce a type I spectral change when

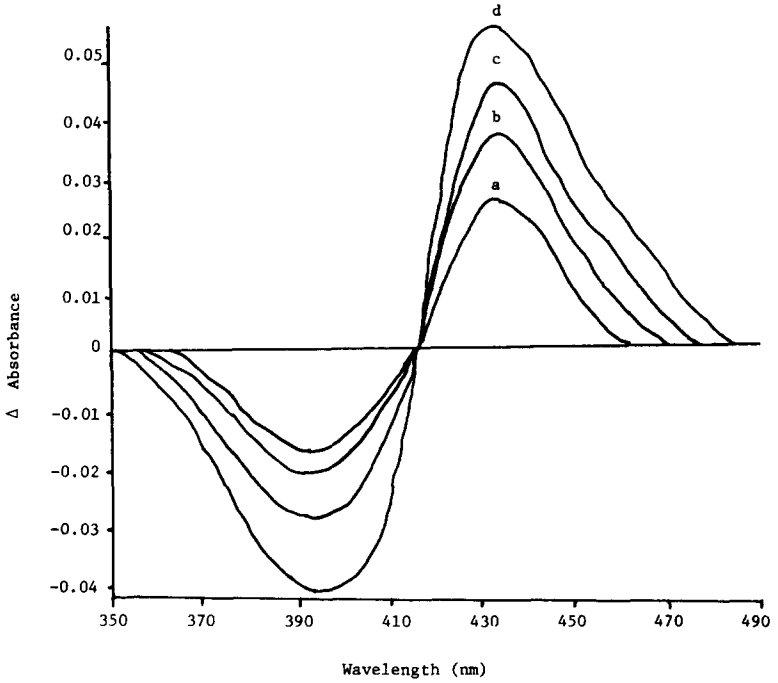

Fig. 1. Substrate binding difference spectra of tranylcypromine with oxidized rat liver microsomes (1.4 mg protein/ml; 0.3 nmole cytochrome P-450/mg protein) at various concentrations: 0.16 mM (a), 0.33 mM (b), 0.66 mM (c), and 1 mM (d).

combined with microsomal cytochrome P-450 [29]. This type of interaction has been proposed to represent the enzyme–substrate complex [15]. Aniline and related amino compounds are bound to the ferric form of cytochrome P-450 as a sixth ligand [15]; similar interaction involved the binding of oxygen and carbon monoxide to the hemoprotein. These compounds induce type II spectral change. These studies prompted us to investigate the spectral interaction of tranylcypromine and cytochrome P-450. Tranylcypromine gives a typical type II spectrum with a half-maximum spectral change value (K_s) of 0.3 mM. These results show that the MAOI is more strongly bound to cytochrome P-450 than are aminopyrine and aniline since their reported K_s values are 1.8 and 2.6 mM, respectively, using rat liver microsomes [29]. Thus, it appears that the inhibitory effect of tranylcypromine on the microsomal oxidative reactions is related to the interaction of the MAOI with cytochrome P-450. This conclusion is reinforced by the fact that tranylcypromine is biotransformed when incubated with microsomal preparations under aerobic conditions (unpublished findings). The preincubation of tranylcypromine for 30 min with the fortified microsomal homogenate, prior to substrate addition, resulted in a decrease in its inhibitory effect of roughly 50% as estimated by the comparison of the K_i values obtained with and without preincubation. It can therefore be concluded that tranylcypromine is itself responsible for the depressive effect and that its metabolic products have less, if any, inhibitory effect. On this basis, tranylcypromine will exert an inhibitory effect only when present in a significant amount on the microsomal membrane.

A recent study reported that tranylcypromine decreased the LD_{50} of pentobarbital, presumably by inhibition of its metabolism [30]. This interaction is clinically relevant because of a reported case of a girl who ingested some tablets of tranylcypromine and died when treated with 1 grain of pentobarbital and 2 grains of secobarbital [31]. The K_i values of tranylcypromine, i.e. the concentration that caused 50% inhibition of the microsomal oxidative reactions, varied from 0.025 to 0.15 mM which corresponds to 3–20 μg/ml of serum of the MAOI. Single dose pharmacokinetic studies of therapeutic doses of tranylcypromine in humans show that the serum or plasma levels of the MAOI vary between 30 and 100 ng/ml [32–34]. However, microgram amounts of the MAOI have been measured in some cases of intoxication [35]. Furthermore, it has been reported that tranylcypromine impairs liver function in humans at therapeutic levels during chronic treatment [36, 37]. Therefore, it is concluded that the depression of microsomal oxidative reactions caused by tranylcypromine is significant enough to be felt *in vivo* both in experimental animals and in humans.

In conclusion, tranylcypromine inhibited competitively the *N*- and *O*-demethylations of aminopyrine and *p*-nitroanisole, respectively, but was a non-competitive inhibitor of the *N*-demethylation of *N,N*-dimethylaniline and aniline hydroxylation. The inhibition constant, K_i, varied between 0.025 and 0.15 mM depending on the substrate used. Preincubation of tranylcypromine for 30 min with the microsomal homogenate prior to substrate addition resulted in a decrease in the inhibitory effect of the MAOI. Tranylcypromine induced a type II spectral change when combined with oxidized cytochrome P-450, with a K_s value to 0.3 mM. It is concluded that the inhibitory effect of tranylcypromine on the microsomal oxidative reactions of rat liver is related to its interaction with cytochrome P-450.

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