High affinity of quinidine for a stereoselective microsomal binding site as determined by a radioreceptor assay[1]

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Summary. The techniques of the radioreceptor binding assay were applied to detect stereoselective binding of quinidine and quinine to a site on human liver microsomes. Binding of ^{3}H-dihydroquinidine was 50% inhibited by 20–100 nM quinidine, while its enantiomer quinine did not displace the ^{3}H-ligand at concentrations up to 500 nM. This stereoselectivity agreed with the affinity values measured by functional enzyme assays of cytochrome P450 activity using sparteine or debrisoquine as substrates.
Key words. Microsome, human liver; radioreceptor assay; stereoselective binding; ^{3}H-dihydroquinidine; quinidine; quinine.

The biotransformation of most drugs involves the cytochrome P450 enzyme system. At least one of the P450 isozymes is genetically polymorphic in human populations[3], implying variability in either the affinity, the density, the catalytic rate (or some combination thereof) of the enzyme for certain drugs. This genetically variable P450 is generally measured by the oxidation of sparteine or debrisoquine[3]. Among the drugs whose fate is known to be affected by this polymorphism are nortriptyline[4], metoprolol[5] and other beta blockers[6,7], as well as perhexiline[8] and phenacetin[9].

We have recently observed[10] that quinidine is a very potent competitive inhibitor of the oxidation of sparteine by human liver preparations, possessing an apparent inhibition constant (K_i) of 60 ± 10 nM (mean ± SD, n = 3). This observation suggests an extremely high affinity of quinidine for this genetically variable, human cytochrome P450; we are not aware of affinities of this magnitude between any of the P450 cytochromes and any other substrate. However, most of the published data on exogenous substrates for these enzyme systems refer to synthetic drugs or chemicals which could not have contributed to the establishment of balanced genetic polymorphisms of drug metabolizing enzymes. Hence, the high affinity of the natural substrate quinidine for a member of the family of cytochromes P450 deserves attention, and an evaluation of the interaction by independent means appeared to be desirable.

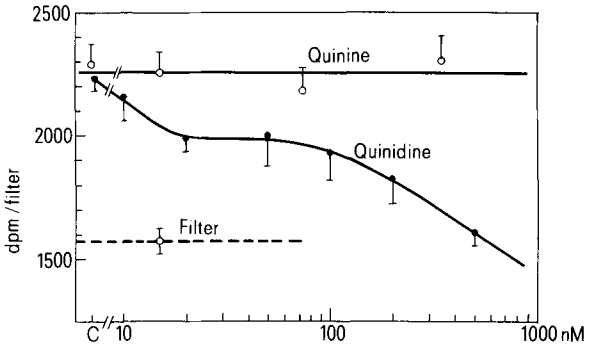
Quinine, the enantiomer of quinidine, is a less potent competitive inhibitor of sparteine metabolism, having a K_i of 15 μM, a value in the range common for most drugs oxidized by this enzyme[11–13]. We have found that this stereoselectivity also holds for the inhibition of debrisoquine 4-hydroxylase, where the K_i for quinidine was 30 nM and that for quinine was 10 μM (Dr M. Nakano, department of pharmacology, personal communication). The hydroxylation of debrisoquine is mediated by the same P450 enzyme which oxidizes sparteine[14–19].

The stereoselective action of the quinidine/quinine enantiomers thus provided us with one of the essential requirements[20–22] for developing a specific radioreceptor assay for the P450 enzyme. The other requirement was to synthesize a specific ^{3}H-ligand with sufficiently high specific activity to label the P450 sites in the nanomolar concentration range. For this purpose, we chose to use ^{3}H-dihydroquinidine (rather than ^{3}H-quinidine itself), custom-synthesized by Dr C. Filer (New England Nuclear Corp., Boston), based on the precedent that alprenolol and dihydroalprenolol have about equal affinities for beta-adrenoceptors[23,24].

Methods. Human liver (obtained from a kidney donor at the time of transplant) had been stored frozen (−70 °C) as 1-cm slices for 3 years, and over this time, had retained a number of cytochrome P450 activities such as sparteine oxidation, debrisoquine 4-hydroxylation, amobarbital 3′-hydroxylation, p-nitroanisole O-demethylation, aminopyrine N-demethylation, and antipyrine 3-methyl- and 4-hydroxylations. A 9 g liver sample was thawed, minced into one volume ice-cold 1.15% KCl, and homogenized in a Potter-Elvehjem tissue grinder using 10 strokes of a rapidly rotating Teflon pestle. The post-mitochondrial supernatant was obtained by centrifugation of the homogenate at 9000 × g for 20 min at 4 °C (Sorvall RC2–B). The supernatant was further centrifuged at 100,000 × g for 60 min at 3 °C (Beckman L2–65B Ultracentrifuge) to recover the microsomal pellet. This pellet was resuspended to the original 9000 × g supernatant volume with cold 1.15% KCl and recentrifuged for 45 min at 100,000 × g. The resulting washed pellet was stored at −70 °C for no more than 2 weeks. Before use, the microsomal pellet was thawed and resuspended using a Brinkmann Polytron (setting No. 7, 20 sec) in 0.2 M KPO_4 buffer, pH 7.4, to obtain a protein concentration of approximately 200 μg/ml.

The binding of ^{3}H-dihydroquinidine (50 Ci/mmole; ^{3}H atoms saturating the double bond at the C10–C11 position) to human liver microsomes was measured as follows. The incubation tube contained 0.5 ml of ^{3}H-dihydroquinidine (final concentration of 2.8 nM) in potassium phosphate buffer, 0.5 ml solutions of quinidine or quinine (ranging from 10 to 500 nM) in buffer, and 0.5 ml microsome suspension. All assays were done in replicates of 12. After incubating the suspension for 1.5 h at room temperature (20 °C), the contents were vacuum-filtered through glass-fiber filters (Whatman GF/B) using a Titertek apparatus (Skatron A.S., Norway), which permits the simultaneous filtration of 12 samples. The filters were washed for 30 sec with 50 mM Tris-HCl buffer. The bound ^{3}H-dihydroquinidine retained on the filters was monitored in 8 ml Scint-A (Packard Co., Chicago) at an efficiency of 36%.

Results and discussion. As shown in the figure, quinidine inhibited the binding of ^{3}H-dihydroquinidine, while quinine did not. Total binding at 2.8 nM ^{3}H-dihydroquinidine was about 2300 dpm/filter, while binding to the filter was about 1600 dpm/filter; 500 nM quinidine inhibited the binding to the level found on the filter control. Thus, if specific binding was defined as that displaced by 500 nM quinidine, the specific binding was about 30% of the total amount bound. Similar results were obtained when the incubations were done for either 1.5 or 23 h at 8 °C, a temperature at which enzymatic activity would be minimal. Efforts to consume any native NADPH-generating capacity (upon which drug oxidation depends) by pre-incu-



Stereoselective displacement of ^{3}H-dihydroquinidine (2.8 nM) by quinidine but not quinine, the enantiomer of quinidine, from human liver microsomes. Total binding of the ^{3}H-ligand was done at negligible concentrations (C) of quinine and quinidine (10 pM). Each point for quinidine in this representative experiment indicates the mean (± SEM) for 12 replicates; 24 replicates for quinine. Room temperature (20 °C).

bating the microsomal suspensions with antipyrine, were found to be unnecessary. Antipyrine is not oxidized by the P450 variant responsible for the oxidations of sparteine and debrisoquine[11, 18, 25].

Radioreceptor assays are generally best for ligands having a dissociation constant, or K_D, less than 5 nM[20–22], permitting the use of low concentrations of ^{3}H-ligand (below 2 nM) and minimizing non-specific binding. The major difficulty with ^{3}H-dihydroquinidine as a ligand is the K_i value, as estimated by its competitive inhibition of sparteine oxidation, of 40 nM (data not shown). This high value predicted the significant noise problem encountered in the binding of this radioligand and necessitated the use of a large number of replicates. As shown in the figure, the data still had a large standard error which prevented quantitative comparisons between the 2 types of assays. However, the Ki of quinidine estimated from its inhibition of sparteine and debrisoquine oxidations (30–60 nM) is within the concentration range over which quinidine inhibited ^{3}H-dihydroquinidine binding (10–500 nM).

The high signal to noise ratio may have been reduced had a more purified preparation of cytochrome P450 been available to us for use. However, having now obtained a prototype ligand, it is possible to proceed with the design of related ligands having higher oil/water partition coefficients, and thus lower K_D values. Ultimate identification of the binding component of the liver microsomal suspension awaits the synthesis of such a ligand.

These observations indicate that the techniques developed for the in vitro study of drug receptors will be applicable to the assessment of membrane-bound enzymes. Such assessment promises to be useful for the study of genetically-variable components of catalytically-inactive systems, as shown here for a member of the cytochrome P450 family. Furthermore, these observations suggest that the enzymatic handling of natural drug substrates (in contrast to artificial substrates) is worthy of attention.

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Do prostaglandins mediate the somatostatin preventive effect on gastric lesion?

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Summary. The inhibition of endogenous prostaglandin synthesis by indomethacin treatment blocks the somatostatin preventive effect on the gastric lesions induced in a stress model and has no preventive effect on an intragastric distension model.
Key words. Rat; prostaglandins; gastric lesion; intragastric distension model; stress model; indomethacin; somatostatin preventive effect.

Somatostatin is a polypeptide that acts at a gastric level in 2 important ways: inhibition of acid secretion and antiulcer activity. Recently it has been verified[1] that exogenous somatostatin stimulates synthesis and endogenous release of prostaglandin F_2 and that indomethacin (a cyclooxygenase inhibitor) blocks the somatostatin inhibitor effect on gastric acid secretion. These authors suggest that endogenous prostaglandins may be mediators in the somatostatin inhibitor effect on gastric acid secretion. To find out whether prostaglandins are also mediators in the somatostatin antiulcer effect at gastric level we examined the somatostatin activity on a stress gastric lesion model, with or without prior indomethacin treatment. Moreover we have studied this using a gastric lesion model independent of the acid inhibitory properties of the drug tested. In this model mucosal erosions were produced by intragastric distension on a continuously perfused simulated gastric juice.

Material and methods. In the stress model a male Wistar rat (200–250 g) group (n = 20) was immobilized in rigid plastic tubes and kept at 4 ± 1 °C for 3 h. 10 of these animals were treated with somatostatin (initial bolus of 2.8 μg/kg then kept at 10 μg/kg/h, i.v.) and the others treated with the same volume of saline solution. Other rats (group n = 20) were kept under the same conditions after an indomethacin treatment (5 mg/kg, i.m.) 2 h before.

In the intragastric distension model lesions were induced on anaesthetized (urethane 1.6 g/kg, i.m.) male Wistar rats