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QUARTERLY

Glutathione *S*-transferase pi as a target for tricyclic antidepressants in human brain✪

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GST pi, the main glutathione *S*-transferase isoform present in the human brain, was isolated from various regions of the brain and the *in vitro* effect of tricyclic antidepressants on its activity was studied. The results indicated that amitripyline and doxepin — derivatives of dibenzcycloheptadiene, as well as imipramine and clomipramine — derivatives of dibenzazepine, inhibit the activity of GST pi from frontal and parietal cortex, hippocampus and brain stem. All these tricyclics are non-competitive inhibitors of the enzyme with respect to reduced glutathione and non-competitive (amitripyline, doxepin) or uncompetitive (imipramine, clomipramine) with respect to the electrophilic substrate. Their inhibitory effect is reversible and it depends on the chemical structure of the tricyclic antidepressants rather than on the brain localization of the enzyme.

We conclude that the interaction between GST pi and the drugs may reduce their availability in the brain and thus affect their therapeutic activity. On the other hand, tricyclic antidepressants may decrease the efficiency of the enzymatic barrier formed by GST and increase the exposure of brain to toxic electrophiles. Reactive electrophiles not inactivated by GST may contribute in adverse effects caused by these drugs.

According to the biogenic amine hypothesis of mood, a functional increase in the activity of serotonin, norepinephrine and dopamine would result in mood elevation and a decrease of their activity – in depression. Many tricyclic compounds, derivatives of diben-

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Abbreviations: CDNB, 1-chloro-2,4-dinitrobenzene; GSH, glutathione; GST, glutathione *S*-transferase.

zcycloheptadiene and dibenzazepine can block monoamine reuptake from the synaptic cleft. As a consequence, the level of serotonin, norepinephrine and dopamine near their receptors rises and the synaptic transmission speeds up. The tricyclic antidepressant drugs belong to nonselective amine reuptake inhibitors. Despite many adverse effects, tricyclic antidepressants are the primary drugs of choice for severe endogenous depression (Kostowski, 1998). Amitriptyline and imipramine, the two first developed agents, are the most commonly prescribed. Tricyclic antidepressants are well adsorbed, widely distributed in the brain and have relatively long half-lives. They are metabolised mainly by hydroxylation, N-demethylation and glucuronidation in the endoplasmic reticulum (Kostowski, 1998). Because of their lipophilic nature they can easily cross the blood-brain barrier, but it is not known whether they need an intracellular transporter to reach the endoplasmic reticulum. The role of such a carrier may be played by glutathione *S*-transferase.

Glutathione *S*-transferase (GST, EC 2.5.1.18) is the most important biotransformation enzyme that inactivates toxic electrophilic compounds by conjugation with reduced glutathione (Jakoby, 1978). GST isoenzymes are expressed and distributed among mammalian tissues, including brain (Theodore *et al.,* 1985; Board *et al.,* 1990; Ali-Osman *et al.,* 1997). These mostly cytosolic enzymes are of great interest not only in relation to their catalytic activity, but also because they are very effective intracellular binding and transport proteins. They bind a large variety of endo- and exogenic hydrophobic compounds including bilirubin, heme, steroid hormones and some drugs (Kamisaka *et al.,* 1975; Listowsky *et al.,* 1988; Oakley *et al.,* 1999). As we have shown in our earlier studies, glutathione *S*-transferase isolated from monkey, bovine and pig brain can bind physiologically active amines (catecholamines, histamine, melatonin), some adrenergic agents (phenylephrine, propranolol) and analgesics (paracetamol, salicylamide) (Barańczyk-Kuźma *et al.,* 1992; Barańczyk-Kuźma & Drobisz, 1993; Sawicki *et al.,* 1997; 2001).

In the present work we studied the interaction between tricyclic antidepressants and glutathione *S*-transferase pi isolated from different regions of human brain.

MATERIALS AND METHODS

Chemicals. DEAE-Sephadex A-25, SDS/PAGE markers for molecular weight 14000–66000 and standard glutathione *S*-transferase from human placenta (GST pi) were from Sigma. Polyclonal rabbit antibody specific for GST pi was from Novocastra. Reagents for polyacrylamide gel electrophoresis and ampholine (pH 5–8), were from Bio-Rad. Tricyclic antidepressants — amitriptyline, doxepin, imipramine and clomipramine — were from ICN Pharm. Inc. All other chemicals were of the highest grade commercially available.

GST pi isolation, identification and assay. GST pi was isolated from human brain obtained from autopsy, 30 h after death. Parietal cortex, frontal cortex, hippocampus and brain stem were separated and stored at –80°C. After thawing they were homogenized in cold 10 mM sodium phosphate buffer, pH 7.6, containing 0.25 M sucrose and centrifuged for 15 min at 15000 × ***g***. The supernatant was centrifuged again for 60 min at 100000 × ***g***. The pellet was discarded and supernatant applied on a DEAE-Sephadex A-25 column, as described previously (Sawicki *et al.,* 1997). The main GST isoform, non-adsorbed on the column, was used for further studies. It contained the majority (60–80%) of the total GST activity obtained from the column. All steps of the procedure were performed at 0–4°C.

The isoelectric point of this isoform was determined on a Rotofor Prep-Cell 491, Bio-Rad

according to the manufacturer's instruction. Western blotting detection was performed using rabbit anti-GST pi polyclonal antibody. Glutathione *S*-transferase from human placenta (GST pi) was used as a standard.

GST activity was measured according to Habig *et al.* (1974) using 1 mM reduced glutathione (GSH) and 1 mM 1-chloro-2,4-dinitrobenzene (CDNB) as an electrophilic substrate. Protein was determined according to Bradford (1976), with bovine serum albumin as a standard. GST specific activity was expressed in μmol/min per mg protein.

Studies were approved by the Ethics Committee on Human Experiments at the Medical University of Warsaw.

Studies on the effect of tricyclic antidepressants on GST pi activity. Samples containing enzyme, 100 mM sodium phosphate buffer, pH 6.5, and the drug – amitriptyline, doxepin, imipramine or clomipramine (Fig. 1)

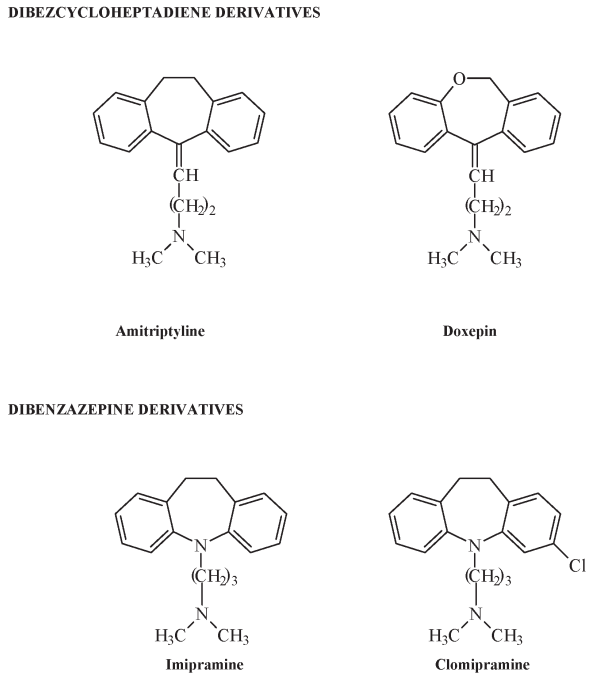

Figure 1. Chemical structure of tricyclic antidepressants.

– were preincubated for 10 min at 37°C, cooled to 4°C, and assayed for GST activity. Blanks without the enzyme were assayed for each concentration of the compound tested. The activity after preincubation without the drug was taken as 100%.

The type of inhibition was determined from double reciprocal plots according to Lineweaver-Burk without preincubation. In inactivation/reactivation studies, the enzyme was treated (without preincubation) with two different concentrations of the drug, then dialyzed for 3 h against 10 mM sodium phosphate buffer, pH 7.6, and assayed for GST activity. The control samples, without the drug tested, were dialyzed separately. Their activity was taken as 100%.

RESULTS

Identification of GST pi

The isoelectric point of human brain GST separated by DEAE-Sephadex A-25 was 4.6 – the same as the pI of the so called placental isoform GST pi. In Western blotting analysis the enzyme isolated from all studied regions of human brain was recognized by antibody raised against GST pi (Fig. 2).

Effect of tricyclic antidepressants on GST pi activity

Derivatives of dibenzcycloheptadiene – amitriptyline and doxepin, as well as derivatives of dibenzazepine – imipramine and clomipramine inhibited the activity of glutathione *S*-transferase pi from all studied regions of human brain (Fig. 3). The strongest inhibitory effect was exerted by amitriptyline and clomipramine. At a 5 mM concentration of these drugs GST pi lost from 50 to 70% of its initial activity, whereas in the presence of doxepin or imipramine only about 30%. The inhibitory effect of these drugs, with the exception of clomipramine, was similar in various regions of the brain. Clomipramine inhibited GST pi from parietal cortex and brain stem more effectively than

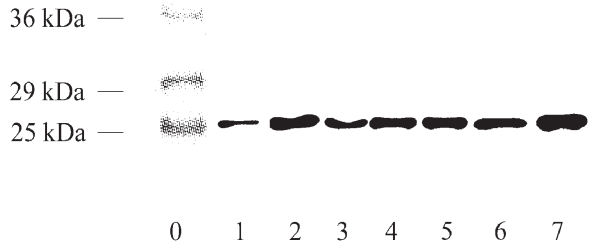

Figure 2. Western blot analysis of GST pi from human brain.

Lane 0 — molecular mass markers; lane 1 — standard GST pi (100 ng); lanes 2, 3, 4 and 6 — 100 000 × ***g*** supernatant from frontal cortex, parietal cortex, hippocampus and brain stem, respectively (4 μg protein); lanes 5 and 7 — GST pi isolated by DEAE-Sephadex from hippocampus and brain stem (4 μg protein).

from parietal cortex and hippocampus (Fig. 3).

Kinetic studies

All the drugs showed the noncompetitive type of inhibition with respect to glutathione (GSH) as the variable substrate (Fig. 4). At variable concentrations of CDNB, the electrophilic substrate, both derivatives of dibenzcycloheptadiene showed the noncompetitive type of inhibition (Fig. 5), whereas both derivatives of dibenzazepine, the uncompetitive type of inhibition (Fig. 6). When the enzyme inactivated by either drug was dia-

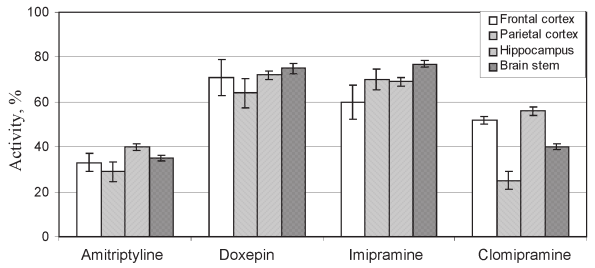

Figure 3. Effect of tricyclic antidepressants on GST pi from different regions of human brain.

GST activity was determined after 10 min preincubation of the enzyme with 5 mM drug, as described in Materials and Methods. Each value is the mean ±S.D. of four experiments, performed in triplicates.

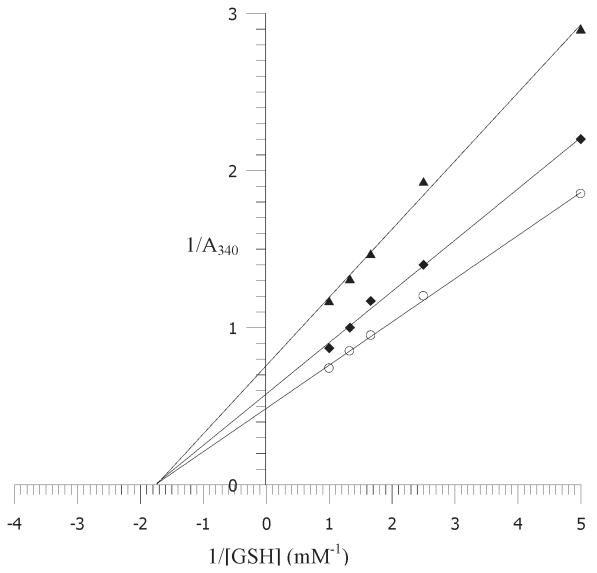

Figure 4. Inhibition type of GST pi from human parietal cortex.

Activity was determined with 1 mM CDNB and varying concentrations of GSH, in the absence (o) and presence of 2.5 mM (■) and 5.0 mM (▲) doxepin. Each value is the mean of four determinations.

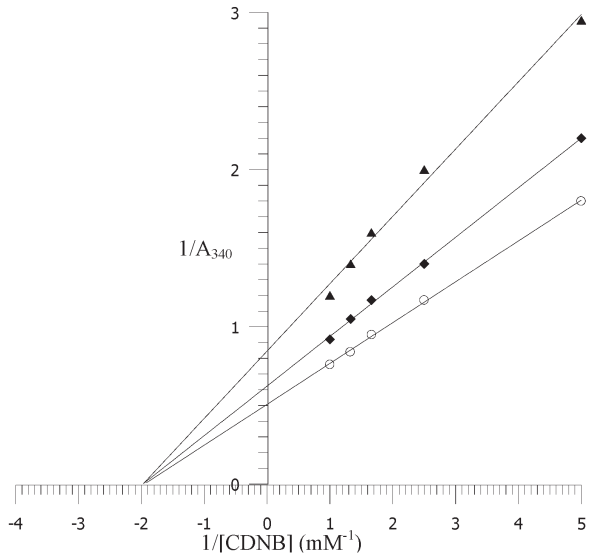

Figure 5. Inhibition type of GST pi from human parietal cortex.

Activity was determined with 1 mM GSH and varying concentrations of CDNB, in the absence (o) and presence of 2.5 mM (■) and 5.0 mM (▲) doxepin. Each value is the mean of four determinations.

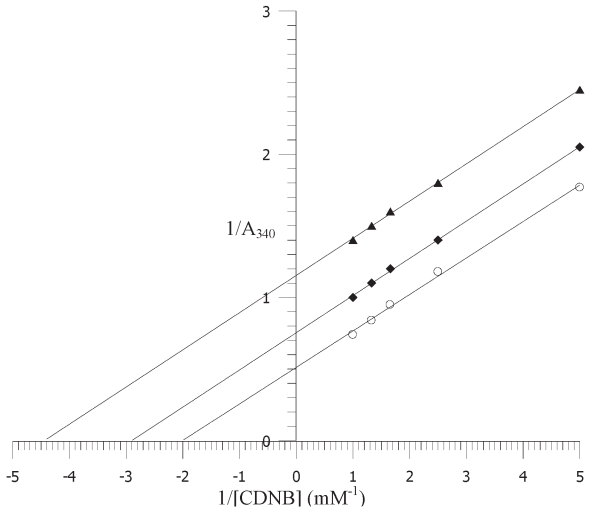

Figure 6. Inhibition type of GST pi from human parietal cortex.

Activity was determined with 1 mM GSH and varying concentrations of CDNB, in the absence (○) and presence of 2.5 mM (■) and 5.0 mM (▲) clomipramine. Each value is the mean of four determinations.

lyzed and subsequently assayed for GST activity, a significant reactivation was observed (Table 1).

DISCUSSION

Despite the increasing number of selective agents used in the treatment of depression, tricyclic antidepressants, derivatives of dibenzcycloheptadiene and dibenzazepine, are still in use. It is well known that these drugs are metabolized by many biotransformation enzymes that catalyze reactions of oxidation, hydroxylation, demethylation, acetylation or glucuronidation. In the present work we demonstrated that glutathione *S*-transferase, another biotransformation enzyme, may be involved in the action of tricyclic antidepressants**.** The results indicate that GST pi, the main, acidic (pI 4.6), isoform present in the human brain may be a target for tricyclic antidepressants. The drugs can decrease GST activity in different regions of the brain. There are significant differences between the effectiveness of the drugs. Replacement of oxygen with a carbon atom in dibenzheptadiene ring and addition of a chloride atom to dibenzazepine ring enhance the inhibitory effects expressed by amitriptyline and clomipramine comparing to that of doxepin and imipramine, respectively. The inhibitory effect of the drugs on GST pi obtained from various regions of the brain was similar. Only clomipramine inhibited GST pi from parietal cortex and brain stem more effectively than that from frontal cortex and hippocampus. More studies are needed to explain these differences.

At present it seems that the inhibitory effect of the tricyclics studied depends more on

Table 1. Inactivation by tricyclic antidepressants and reactivation of GST pi

Drug	Concentration (mM)	Activity (%) after inhibition	Activity (%) after inhibition and dialysis
Amitriptyline	5.0	54 ± 4	100
	7.5	33 ± 4	70 ± 2
	10.0	60 ± 2	82 ± 2
Doxepin	15.0	32 ± 4	52 ± 3
	10.0	65 ± 2	100
Imipramine	15.0	22 ± 4	60 ± 4
	5.0	64 ± 3	100
Clomipramine	10.0	20 ± 4	33 ± 3

Brain stem enzyme was treated with indicated concentrations of drugs. GST activity was determined before and after dialysis, as described in Materials and Methods. The activity without the drug tested was taken as 100%. Each value is the mean ±S.D. of two separate experiments, each performed in triplicate.

their chemical structure than on GST pi localization in the brain.

Kinetic studies indicate that GST pi binds the tricyclic antidepressants nonspecifically. All these tricyclics are bound to the effector (ligandin) site of the enzyme (noncompetitive inhibitors), whereas only dibenzazepine derivatives, which are uncompetitive inhibitors, bind to GST before the electrophilic substrate. The physical interaction between GST and the drugs may reduce their availability in the brain and thus affect their therapeutic activity. Since the binding is reversible, the effect may be temporal. On the other hand, tricyclic antidepressants, usually administrated for prolonged periods (months or years) by inhibiting GST may decrease the efficiency of the enzymatic barrier formed by this enzyme and increase the exposure of brain to toxic electrophiles. The reactive electrophiles not inactivated by GST may contribute in adverse effects caused by these drugs.

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