Biochemical Pharmacology, Vol. 43, No. 8, pp. 1865–1868, 1992.
Printed in Great Britain.

0006-2952/92 $5.00 + 0.00

SHORT COMMUNICATIONS

Pharmacological comparison of the rat and guinea-pig cortical high affinity 5-hydroxytryptamine uptake system

(*Received* 6 *September* 1991; *accepted* 11 *February* 1992)

Abstract—The pharmacological characteristics of the high affinity [^{3}H]5-hydroxytryptamine ([^{3}H]5-HT) uptake system were investigated in the cerebral cortex of the rat and guinea-pig. In crude cortical synaptosomal preparations from the rat and guinea-pig, [^{3}H]5-HT accumulated with high affinity (K_m, 72 ± 12 and 57 ± 14 nM for rat and guinea-pig cortical synaptosomal preparation, respectively, mean ± SEM, N = 5) and with a comparable maximum activity (V_{max}, 1.22 ± 0.21 and 0.90 ± 0.19 pmol/min/mg protein for rat and guinea-pig cortical synaptosomal preparation, respectively, mean ± SEM, N = 5). Competition studies employing a range of structurally diverse competing compounds showed that the [^{3}H]5-HT uptake was pharmacologically similar in both preparations. However, citalopram possessed approximately 10-fold weaker affinity to prevent [^{3}H]5-HT uptake in the guinea-pig preparation when compared to the rat and all of the tricyclic antidepressants assessed in the present studies (amitriptyline, nortriptyline, desipramine and imipramine) displayed higher affinity in the guinea-pig preparation when compared to the rat. It is concluded that the high affinity 5-HT uptake systems in the rat and guinea-pig cortex are similar but may not be identical.

Components of the 5-hydroxytryptamine (5-HT*) system have been proposed as targets for the therapeutic management of eating disorders, depression, anxiety, psychosis, cognitive impairment, hypertension and migraine (e.g. Refs 1–3). Although this indoleamine is widely distributed throughout the animal kingdom it is apparent that species differences with respect to certain components of the system occur. For example, one of the 5-HT receptor subtypes, the 5-HT_{1B} receptor, appears to be specific to rats and mice; its "functional equivalent", the 5-HT_{1D} receptor, being found in some other animals species (e.g. guinea-pig) and also man (for review see Ref. 4). In addition, species variation has been ascribed to the 5-HT_3 receptor (e.g. Refs 5–7). Of note is that the guinea-pig 5-HT_3 receptor displays up to three orders of magnitude lower affinity for a range of structurally diverse antagonists compared to those of the rat (e.g. Ref. 7).

Since it would appear that at least two elements of the 5-HT system in the guinea-pig are pharmacologically distinct from those in the rat, we assessed the potential species variation between the rat and guinea-pig high affinity 5-HT uptake system. The pharmacology of this latter system has not been reported previously. A preliminary communication of this data has been presented to the British Pharmacological Society [8].

Materials and Methods

Preparation of synaptosomes. Female hooded-Lister rats (250–300 g, Olac) or female guinea-pigs (500–900 g, Dunkin Hartley) were killed by cervical dislocation and the brain rapidly removed over ice. The cerebral cortex was dissected and homogenized in 20 volumes (w/v) 0.32 M sucrose using a glass mortar and teflon pestle homogenizer (clearance 0.11–0.15 mm). The homogenate was centrifuged (1000 *g*, 10 min, 4°) and the supernatant recentrifuged (48,000 *g*, 10 min, 4°). The supernatant was discarded and the pellet gently resuspended in 0.27 M sucrose to form the crude synaptosomal preparation. Protein content was determined by the method of Bradford [9] using bovine serum albumin as the standard.

Assessment of [^{3}H]*5-HT uptake.* To initiate high affinity [^{3}H]5-HT uptake, 250 μL of the crude synaptosomal preparation (or 0.27 M sucrose for filter blanks) were added to pre-incubated test-tubes (in triplicate, 2 min, 37°) containing 650 μL of gassed (95% O_2/5% CO_2) artificial cerebrospinal fluid [aCSF(mM): NaCl 126.6, $NaHCO_3$ 27.4, KCl 2.4, KH_2PO_4 0.49, $CaCl_2$ 1.2, $MgCl_2$ 0.83, Na_2HPO_4 0.49, glucose 7.1, pargyline 0.01] and 100 μL [^{3}H]5-HT in aCSF (final concentration of 8.0–10.7 nM for competition studies or a range of nine concentrations between 2.5 and 200 nM for saturation studies) in the absence and presence of competing compounds. Uptake was allowed to proceed at 37° for 6 min before termination by rapid filtration through Whatman GF/B filters followed by immediate washing for 12 sec with ice-cold aCSF. The radioactivity remaining on the filters was quantified by liquid scintillation spectroscopy at an efficiency of approximately 47%.

Drugs. Amitriptyline (HCl, Sigma), benztropine (mesylate, Research Biochemicals Inc.), citalopram (HBr, Lundbeck), cocaine (HCl, May and Baker), desipramine (HCl, Sigma), fenfluramine (HCl, Sigma), fluoxetine (HCl, Lilly), GBR12909 (1-[2-[bis(4-fluorophenyl)methoxy] ethyl]-4-[3-phenylpropyl]piperazine.HCl, Research Biochemicals Inc.), hemicholinium-3 (Sigma), imipramine (HCl, Sigma), mazindol (RBI), nomifensine (Research Biochemicals Inc.), 8-OHDPAT ((±)-8-hydroxydipropylaminotetralin·HBr, Research Biochemicals Inc.), and paroxetine (HCl, SmithKline Beecham) were dissolved in a minimum volume of water and diluted with aCSF. [^{3}H]5-HT (12.4–25.4 Ci/mmol, Amersham or NEN) was supplied in 98/2 water/ethanol and was diluted with aCSF.

Results

Saturation studies. [^{3}H]5-HT uptake saturation experiments with crude synaptosomal preparations from both the rat and guinea-pig cortex indicated that specific [^{3}H]5-HT uptake (defined by citalopram, 10 μM) was of high affinity and associated with a saturable homogenous population of uptake sites (K_m, 72 ± 12 and 57 ± 14 nM; V_{max}, 1.22 ± 0.21 and 0.90 ± 0.19 pmol/min/mg protein for rat and guinea-

* Abbreviations: 5-HT, 5-hydroxytryptamine; GBR12909, 1-[2-[bis(4-fluorophenyl)methoxy]ethyl]-4-[3-phenylpropyl]piperazine·HCl; 8-OHDPAT, (±)-8-hydroxydipropylaminotetralin·HBr; aCSF, artificial cerebral spinal fluid.

Table 1. Comparison of the affinities of various compounds to compete with [^{3}H]5-HT for the high affinity 5-HT uptake in rat and guinea-pig cortical crude synaptosomal preparations

Drug	pK_i Rat	pK_i Guinea-pig
Paroxetine	8.12 ± 0.04	7.99 ± 0.13
Citalopram	7.71 ± 0.13	6.81 ± 0.13*
Fluoxetine	6.64 ± 0.10	6.70 ± 0.06
Imipramine	6.54 ± 0.06	7.28 ± 0.04‡
Mazindol	6.38 ± 0.10	6.08 ± 0.12
Cocaine	6.25 ± 0.10	6.77 ± 0.00
Amitriptyline	6.39 ± 0.08	7.07 ± 0.03†
8-OHDPAT	6.14 ± 0.04	6.07 ± 0.00
Nortriptyline	6.04 ± 0.06	6.54 ± 0.05†
GBR12909	5.90 ± 0.08	5.55 ± 0.04
Fenfluramine	5.75 ± 0.30	5.84 ± 0.27
Desipramine	5.78 ± 0.08	6.23 ± 0.07†
Hemicholinium-3	5.57 ± 0.10	5.00 ± 0.12
Benztropine	4.90 ± 0.09	5.12 ± 0.05
Nomifensine	4.86 ± 0.23	5.15 ± 0.09

pK_i Values were calculated with the Cheng–Prussoff equation: $pK_i = -\log_{10}(IC_{50}/(1 + ([L]/K_m)))$, where IC_{50} is the molar concentration of the competing compound to inhibit 50% of the high affinity uptake, [L] is the molar concentration of [^{3}H]5-HT in the incubation medium and K_m the molar Michaelis constant derived from saturation experiments.

Each value represents the mean ± SEM, N = 4–6. Significant difference between rat and guinea-pig
*P < 0.05, †P < 0.01, ‡P < 0.001 (Student's *t*-test).

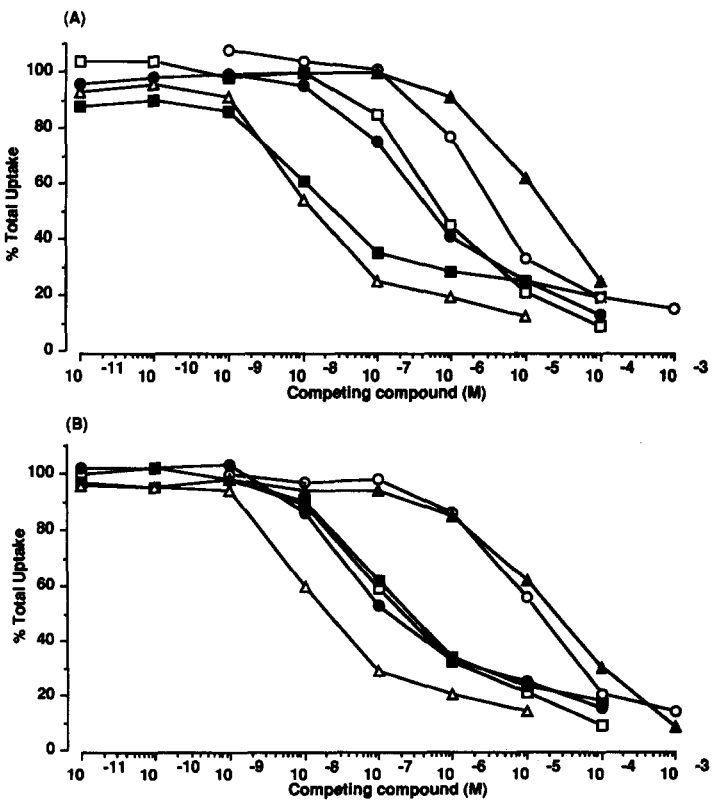

Fig. 1. Ability of various compounds to inhibit [^{3}H]5-HT uptake into synaptosomes prepared from rat (A) and guinea-pig (B) cerebral cortex. Data represent the means from three individual experiments (SE in the range of 0.3–25% of the mean value). Amitriptyline (□), citalopram (■), hemicholinium-3 (○), imipramine (●), nomifensine (▲), paroxetine (△).

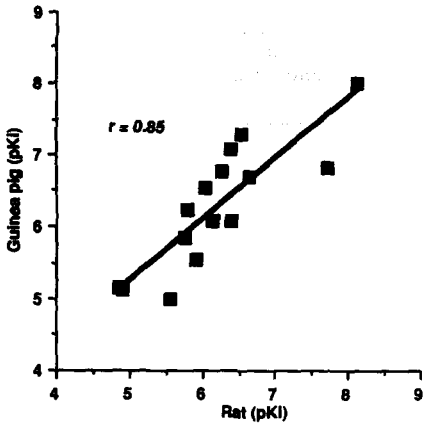

Fig. 2. Correlation of the affinities of various compounds to inhibit [^{3}H]5-HT uptake into synaptosomes prepared from rat and guinea-pig cerebral cortex (data taken from Table 1). r, linear correlation coefficient.

pig cortical synaptosomal preparation, respectively, mean ± SEM, N = 5).

Competition studies. In both the guinea-pig and rat cortical crude synaptosomal preparations, [^{3}H]5-HT uptake was inhibited by up to 80–90% by a range of compounds with affinities (pK_i) ranging from 8.12 to 4.86 (Table 1, Fig. 1). The affinities of competing compounds between the two preparations were similar although some differences were apparent (Fig. 2). For instance, citalopram was found to be approximately 10-fold more potent in inhibiting [^{3}H]-5-HT uptake in the rat preparation compared to that of the guinea-pig, whilst amitryptyline, nortriptyline, desipramine and imipramine were approximately 3–6-fold more potent in the guinea-pig preparation compared to that of the rat (Table 1, Fig. 1).

Discussion

In the present studies [^{3}H]5-HT was shown to accumulate into crude cortical synaptosomal preparations from the rat and guinea-pig with similar affinity (K_m) and with a comparable maximum activity (V_{max}). Furthermore, the saturation characteristics of the [^{3}H]5-HT uptake in the rat cortical preparation were comparable with previous studies (e.g. Refs. 10, 11).

The pharmacology of high affinity 5-HT uptake in the rat was similar to that in the guinea-pig. 5-HT uptake in the rat brain has previously been the subject of detailed pharmacological characterization, and the present results are comparable with previous studies. Thus, the potent and selective 5-HT uptake inhibitor, paroxetine, was shown to compete at nanomolar concentrations whilst compounds which have primary actions at additional neurotransmitter/metabolite high affinity uptake channels (e.g. GBR12909, dopamine uptake blocker; desipramine, noradrenaline uptake blocker; hemicholinium-3, choline uptake blocker) were considerably weaker (e.g. Refs 11–17).

There were, however, some compounds which displayed small, but consistant differences in affinity to prevent the high affinity uptake of [^{3}H]5-HT in the rat and guinea-pig cortical preparations. Thus, the relatively potent and selective 5-HT uptake blocker citalopram [15] was approximately 10-fold weaker to prevent [^{3}H]5-HT uptake in the guinea-pig preparation when compared to the rat, whilst all of the tricyclic antidepressants (dibenzazepines and dibenzcycloheptenes) assessed in the present studies (amitryptyline, nortriptyline, desipramine and imipramine) displayed some 3–6-fold higher affinity to inhibit [^{3}H]5-HT uptake in the guinea-pig preparation when compared to the rat. It is not known, however, whether these small affinity differences reflect a difference in affinity for the amine recognition site or for the functioning of the amine carrier.

By direct comparison, it remains to be determined which, if either, of the high affinity 5-HT uptake systems described in the present studies resembles that present within the human CNS. It is appreciated, however, that due to the scarcity of "fresh" human brain tissue, competition studies with a selective radioligand for the 5-HT uptake system (e.g. [^{3}H]paroxetine) in post mortem human brain tissue are likely to provide a more practical assessment of the pharmacology of the human 5-HT uptake system. It may be important to note, however, that direct pharmacological comparison of the rat and human platelet high affinity 5-HT uptake systems has identified differences in their pharmacology [18].

In summary, the present studies have shown that while the pharmacology of the rat and guinea-pig cortical high affinity 5-HT uptake systems is similar, some compounds have been identified which display a small degree of selectivity which tentatively indicates that they may not be identical.

Acknowledgements—We are most grateful to Drs T. P. Blackburn (Smithkline Beecham) and J. Arnt (Lundbeck) for the generous gifts of paroxetine and citalopram, respectively.

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Biochemical Pharmacology, Vol. 43, No. 8, pp. 1868–1871, 1992.
Printed in Great Britain.

0006-2952/92 $5.00 + 0.00

Cytochrome P450-dependent mixed-function oxidase and glutathione *S*-transferase activities in spontaneous obesity-diabetes

(*Received* 10 *January* 1992; *accepted* 7 *February* 1992)

Abstract—The effect of non-insulin-dependent diabetes on the hepatic microsomal cytochrome P450-dependent mixed-function oxidase system and on cytosolic glutathione *S*-transferase activity was determined using the spontaneously obese-diabetic (ob/ob) mouse model. The activities of the xenobiotic-metabolizing cytochrome P450 proteins were monitored by the use of chemical probes. Non-insulin-dependent diabetes did not influence the hepatic metabolism of substrates associated with the P450 I, IIB, IIE, III and IV families of cytochromes. In contrast, cytosolic glutathione *S*-transferase activity was markedly reduced and glutathione levels were significantly lowered. These findings raise the possibility that patients suffering from this disease may be more susceptible to chemicals that rely on glutathione conjugation for their deactivation.

The cytochrome P450-dependent monooxygenases are probably the most important oxidase system, being responsible for the oxidation of endogenous substrates such as fatty acids, steroids, eicosanoids and vitamins, and also for the deactivation and detoxication of xenobiotics that gain entry into the living organism. It achieves this broad specificity by existing as a number of structurally distinct families of proteins, each with characteristic substrate specificity [1]. Certain families, such as the P450 I and to a lesser extent the P450 IIE, have the propensity to metabolize chemicals at positions, the oxidation of which results in the formation of reactive electrophilic species that interact with DNA and other important molecules giving rise to toxicity/carcinogenicity [2]. Clearly any change in the levels and/or composition of cytochrome P450 proteins will have a consequence for the way a living organism deals with a chemical and whether toxicity will ensue.

In the early 60s [3] it was demonstrated that chemically induced type I, insulin-dependent diabetes modified the ability of hepatic preparations to metabolize a number of drugs and this was substantiated and extended by many other workers, employing different model substrates [4]. More recently it was established that the mechanism involved was a profound effect of the disease on hepatic cytochrome P450 proteins that participate in the metabolism of both endogenous and exogenous substrates [5–8]. The outcome of the alterations in cytochrome P450 composition and levels was that the diabetic animal was more susceptible to the toxicity of chemicals such as carbon tetrachloride and other chemical toxins [9] and, moreover, hepatic preparations from diabetic animals were significantly more effective in converting various promutagens, including nitrosamines and aromatic and heterocyclic amines, to mutagenic species in the Ames mutagenicity assay [5, 10, 11]. The present study was undertaken to investigate