British Journal of Pharmacology (2001) 134, 807–814

www.nature.com/bjp

Intracellular distribution of psychotropic drugs in the grey and white matter of the brain: the role of lysosomal trapping

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1 Since the brain is not a homogenous organ (i.e. the phospholipid pattern and density of lysosomes may vary in its different regions), in the present study we examined the uptake of psychotropic drugs by vertically cut slices of whole brain, grey (cerebral cortex) and white (corpus callosum, internal capsule) matter of the brain and by neuronal and astroglial cell cultures.

2 Moreover, we assessed the contribution of lysosomal trapping to total drug uptake (total uptake = lysosomal trapping + phospholipid binding) by tissue slices or cells conducting experiments in the presence and absence of 'lysosomal inhibitors', i.e., the lysosomotropic compound ammonium chloride (20 mM) or the Na^+/H^+-ionophore monensin (10 μM), which elevated the internal pH of lysosomes. The initial concentration of psychotropic drug in the incubation medium was 5 μM.

3 Both total uptake and lysosomal trapping of the antidepressants investigated (imipramine, amitriptyline, fluoxetine, sertraline) and neuroleptics (promazine, perazine, thioridazine) were higher in the grey matter and neurones than in the white matter and astrocytes, respectively. Lysosomal trapping of the psychotropics occurred mainly in neurones where thioridazine sertraline and perazine showed the highest degree of lysosomotropism.

4 Distribution interactions between antidepressants and neuroleptics took place in neurones *via* mutual inhibition of lysosomal trapping of drugs.

5 A differential number of neuronal and glial cells in the brain may mask the lysosomal trapping and the distribution interactions of less potent lysosomotropic drugs in vertically cut brain slices.

6 A reduction (*via* a distribution interaction) in the concentration of psychotropics in lysosomes (depot), which leads to an increase in their level in membranes and tissue fluids, may intensify the pharmacological action of the combined drugs.

British Journal of Pharmacology (2001) **134,** 807–814

Keywords: Antidepressants; neuroleptics; interaction; lysosomal trapping; tissue binding; brain; grey matter; white matter; neurones; astrocytes

Abbreviations: AMI, amitriptyline; CGM, complete growing medium; FLX, fluoxetine; HBSS, Hank's balanced salt solution; IMI, imipramine; PER, perazine; PZ, promazine; SRT, sertraline; THIOR, thioridazine

Introduction

After de Duve *et al*. (1955) had characterized rat liver lysosomes, these cellular organelles and their associated enzymes have now been detected in many organs and tissues (Hirsh 1968; 1969; de Duve, 1974; Freysz *et al*., 1979). On the basis of their studies with the dibasic drug chloroquine, MacIntyre & Cutler (1988a, b) proposed lysosomal trapping as a major factor determining distribution of basic amphiphilic drugs. Weak bases in their non-ionized state permeate membranes and accumulate in the acidic interior of lysosomes where they are protonated and thus become unable to diffuse back into the cytosol (de Duve *et al*., 1974; Ohkuma & Poole, 1978). This process is saturable, energy-dependent and requires cellular integrity. When two basic lipophilic drugs are simultaneously trapped by lysosomes, a bigger increase in the internal pH of the organella takes place compared to that when only one drug is trapped from the cytosol. Consequently, the process of lysosomal trapping is more efficiently saturated and may cause a decrease in tissue uptake of both drugs. At a cellular level, this would indicate a decrease in the intralysosomal drug level and an increase in its membrane and cytosol concentrations.

Our earlier studies showed that lysosomal trapping was an important mechanism of distribution and pharmacokinetic interactions between basic lipophilic psychotropics. However, the tissue distribution of the aliphatic-type phenothiazine neuroleptic promazine, tricyclic antidepressants and selective serotonin reuptake inhibitors depended more on phospholipid binding than on lysosomal trapping (Daniel & Wójcikowski, 1997a, b), whereas, in the case of the piperidine-type phenothiazine neuroleptic thioridazine and the piperazine-type perazine, lysosomal trapping was as important for the tissue uptake as was phospholipid binding (Daniel & Wójcikowski, 1999a, b). The above lysosomotropic properties of psychotropics were especially pronounced in lysosome-rich organs such as lungs, liver and kidneys but less so in the brain.

However, the structure of the brain is not homogenous. Therefore the contribution of the two mechanisms (lysosomal trapping and tissue binding) to total drug uptake may vary in different areas of the brain. Mammalian central nervous system contains three main types of cells, namely neurones, astroglia, and oligodendroglia, which form the grey and white matter of

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the brain. Differentiation between the grey and white matter is related mainly to the presence of neuronal cell bodies and unmyelinated fibres in the grey matter, and of largely myelinated fibres and a high proportion of glial cells in the white matter. Astrocytes found in the grey matter are located in association with neuronal cell bodies, dendrites, capilaries, and especially synapses; moreover, they not only play a 'supporting role' but also modulate interneuronal synaptic transmission. A number of receptor types, such as nicotinic cholinergic, glutamatergic, opioid, dopaminergic, $GABA_A$, glycine, serotoninergic, β-adrenergic and purinergic, are expressed on astrocytes (Hösli & Hösli, 1993; Tembumi & Jacob, 2001). Hirsch (1968; 1969) showed that the activity of the lysosomal marker enzyme, acid phosphatase, in the grey matter was 2–10 times higher than in the white matter of the brain in man, monkey, rat and guinea-pig. Freysz *et al*. (1979) found that the specific activities of lysosomal hydrolases (arylsulfatases A, B, and C, β-galactosidases, and acid phosphatase) in rabbit neurones were 10–25 times higher than in the oligodendroglia, and five times higher than in the astroglia.

The lipid content of the brain is considerably higher than in other organs (Moor *et al*., 1988) and experimental evidence gathered in various animals and humans points to a differential lipid content and composition in the grey and the white matters of the brain (Chavko *et al*., 1993; Söderberg *et al*., 1990; Zhang *et al*., 1996). Total lipid content in the white matter is twice as high as in the grey matter. The highest concentration of lipids is observed in the medulla oblongata and, in descending order, in the hippocampus, cerebellum, basal ganglia, and cortex (Chavko *et al*., 1993). Areas with a higher total lipid content have higher levels of cerebrosides and sulfatides and lower percentages of phosphatidylcholine and phosphatidylinositol. The differences in lipid content and composition between regions are attributable to the relative presence of the grey/white matter. Higher concentrations of cerebrosides, sulfatides and cholesterol, as well as phosphatidylethanolamine/phosphatidylcholine ratio found in the white matter are characteristics of myelin which is present mainly in the white matter. Thus the binding of drugs to lipids may vary amongst brain areas.

Since the brain is not a homogenous organ, i.e. the phospholipid pattern and density of lysosomes may vary in its different regions, the present study was designed to examine the total uptake of psychotropic drugs by vertically cut slices of rat whole brain, the grey (cerebral cortex) and the white (corpus callosum, internal capsule) matters of the brain, as well as by primary neuronal and astroglial cell cultures. Moreover, an attempt was made to assess the contribution of lysosomal trapping to the total drug uptake by tissue slices or cell cultures by conducting the experiments in the absence of lysosomal trapping and presence of phospholipid binding, i.e., in the presence of 'lysosomal inhibitors' such as the lysosomotropic compound ammonium chloride or the Na^+/H^+-ionophore monensin, which elevated the intralysosomal pH.

Methods

Animals

The experiment was carried out on male adult Wistar rats (240–260 g) kept under standard laboratory conditions. The animals were fed *ad libitum* on standard granulated food and had free access to tap water.

Neuronal and astroglial cell cultures

Primary neuronal cultures were prepared from 16-day-old foetuses of Wistar rats, according to a slightly modified method of Bird & James (1973). Briefly, the foetuses were removed and put into ice-cold Hank's balanced salt solution (HBSS). Heads were cut off and placed in another dish containing HBSS and glucose (1% w v^{-1}). Brain cortices were separated in a dish containing Eagle's medium. The tissue was minced and passed through 120 μm- and 30 μm-pore-size nylon meshes, and the suspension was centrifuged at 1000 × *g* for 5 min. The pellet so obtained was dispersed in a complete growing medium (CGM) containing Eagle's medium supplemented with a 10% v v^{-1} foetal calf serum, 2 mM L-glutamine and antibiotics (penicillin, 50 U ml^{-1}, and streptomycin, 50 μg ml^{-1}). Finally, the cells were plated on 24-well culture dishes (5×10^5 $well^{-1}$) precoated with poly-L-lysine, 2.5 μg cm^{-2}. The cultures were incubated at 37°C in an atmosphere of 95% air and 5% CO_2, at approximately 100% humidity. After 48 h, the cells were treated with cytosine-β-arabinofuranoside (10 μM) in order to inhibit multiplication of non-neuronal cells. After another 48 h, medium containing cytosine-β-arabinofuranoside was replaced with a fresh CGM, which was then changed every 3 days. The whole procedure was performed under sterile conditions. The experiment was carried out on day 7 of incubation. The contents of each well (about 0.04 mg protein ml^{-1}) was used as a separate sample.

Primary astroglial cultures from postnatal 1-day-old rat pups were prepared basically as described by McCarthy & de Vellis (1980). In brief, the animals were killed by decapitation and their brains were removed and placed in ice-cold HBSS containing (1% w v^{-1}) glucose. Cerebral hemispheres were freed of the meninges and mechanically dissociated, at first with a fire-polished Pasteur pipette and then by passing the tissue pieces sequentially through sterile 135 μm- and 35 μm-pore-size nylon sieves into CGM. Cells were centrifuged (1000 × *g*, 10 min), suspended in CGM and put into 175 cm^3 tissue culture flasks precoated with poly-L-lysine, 2.5 μg cm^{-2}. After the cells had been cultured for 7 days, oligodendrocytes were shaken off and the remaining astrocytes were trypsinized, centrifuged, washed with HBSS, dispersed in CGM and plated on 24-well culture dishes (5×10^5 $well^{-1}$) precoated with poly-L-lysine, 2.5 μg cm^{-2}. The cultures were incubated at 37°C in an atmosphere of 95% air and 5% CO_2, and at approximately 100% humidity. The experiment was carried out on day 10 after the passage of astrocytes from the flasks to the plates. The contents of each well (about 0.13 mg protein ml^{-1}) was used as a separate sample.

Determination of the uptake of psychotropic drugs by tissue slices and cell cultures

The uptake of psychotropic drugs by vertically cut slices of the whole brain, the grey (cerebral cortex) and the white (corpus callosum, internal capsule) matters of the brain, as well as by neuronal and astrocyte cell cultures was examined. The experiment was carried out according to the previously

developed and described method of Daniel *et al.* (1995). After decapitation, the brain was isolated (the cerebellum and brain stem were discarded) and placed in ice-cold Krebs-Henseleit buffer (pH 7.4), saturated with carbogen (95% O_2/5%CO_2) at 37°C. Tissue slices (50 mg, 1 mm thick) were prepared using a McIlwain chopper and were incubated in 1 ml of the buffer containing a neuroleptic and/or an antidepressant at an initial concentration of 5 μM each. That initial drug concentration allowed the attainment of a steady-state medium and tissue concentration at a range similar to that of therapeutic concentrations in the plasma and tissues, respectively, *in vivo* (Daniel *et al.*, 2000). Incubations were allowed to proceed for 1 h in a shaking water bath at 37°C. In the case of cell cultures, neurones (approximately 0.04 mg of protein per sample) or astrocytes (approximately 0.13 mg of protein per sample) were incubated in 1 ml of the CGM for 30 min in an atmosphere of carbogen at 37°C. In the case of both neuroleptics and antidepressants, the steady-state distribution was reached within 30 min (cell cultures) or 1 h (tissue slices) (Novelli *et al.*, 1987; Daniel & Wójcikowski, 1997a).

In order to determine the contribution of lysosomal trapping to the total tissue uptake of psychotropics and to the distribution interactions between antidepressants and neuroleptics, the experiment was carried out in the presence or absence of NH_4Cl (20 mM) or monensin (10 μM). Since both ammonium chloride (a lysosomotropic compound) and monensin (Na^+/H^+-ionophore) are commonly recognized as fast and efficient 'lysosomal inhibitors' (de Duve *et al.*, 1974; Ohkuma & Poole, 1978; MacIntyre & Cutler, 1988a), it may be assumed that under our experimental conditions the 'lysosomal inhibitors' used at optimum concentrations totally abolished the drug uptake into acidic compartments. In the experimental model used, those concentrations produced a maximal effect, i.e. maximum inhibition of the drug uptake (Daniel & Wójcikowski, 1999a, b). The concentration of drug taken up by tissue slices or cell cultures was calculated from the difference between the initial concentration (5 μM) and the respective value found after incubation in the medium. There was no statistically significant non-specific drug binding by test-tubes or culture dishes at the concentration used.

Drug assay in the incubation medium

After extraction from the incubation medium, the concentrations of neuroleptics (promazine, perazine, thioridazine) and antidepressants (imipramine, amitriptyline, fluoxetine, sertraline) were measured using LaChrom high performance liquid chromatography system (Merck-Hitachi), equipped with a u.v. detector, an L-7100 pump and a D-7000 System Manager, according to previously described procedures (Daniel & Wójcikowski, 1997b; 1999a, b). The analytical column econosphere CN (5 μm, 4.6 × 250 mm) from Alltech (Carnforth, U.K.) was used. The mobile phase consisted of 0.01 M phosphate buffer (pH = 6.5), acetonitrile and methanol (and triethylamine 0.03%) in the proportion 60 : 120 : 40 (v v^{-1}). The flow rate was 1.2 ml min^{-1}. The absorbance of the majority of tested drugs was measured at a wavelength of 254 nm. In the case of thioridazine + imipramine combination, the proportion of the mobile phase components was 60 : 40 : 40 (v v^{-1}), the flow rate 1 ml min^{-1}, the analytical wavelength 264 nm. Retention times were: 4.30 min for sertraline, 5.31 min for amitriptyline, 5.70 min for perazine, 6.26 min for fluoxetine, 10.66 min for thioridazine and 12.31 for imipramine.

Drugs and chemicals

The following drugs were used: promazine hydrochloride (Polfa, Jelenia Góra, Poland), perazine dimaleate (Labor, Wrocław, Poland), thioridazine hydrochloride and imipramine hydrochloride (Polfa, Jelenia Góra, Poland), amitriptyline (H. Lundbeck A/S, Copenhagen, Denmark), fluoxetine hydrochloride (Eli Lilly, Indianapolis, U.S.A.), sertraline hydrochloride (Pfizer, Brussels, Belgium).

The following chemicals were used for cell cultures: cytosine-β-D-arabino-furanoside, poly-L-lysine, L-glutamine, trypsin, penicillin, streptomycin (SIGMA, St. Louis, U.S.A.), calf foetal serum (Hungarpol, Budapest, Hungary), Hank's balanced salt solution, Eagle's medium (Serum and Vaccine Works, Lublin, Poland), monensin sodium salt was provided by SIGMA (Deisenhofen, Germany).

Statistics

The results obtained were elaborated statistically using Dunnett's or Student's *t*-test. Experiments in which only two groups, i.e. the control and a test group were used, were estimated statistically using Student's *t*-test. Experiments using the control and more than one test group were elaborated statistically by analysis of variance, followed by Dunnett's test.

Results

The uptake of psychotropic drugs by vertically cut brain slices

As shown in Figure 1, under steady-state conditions the highest tissue uptake, expressed as an absolute tissue concentration (nmol 50 mg of $tissue^{-1}$) (Figure 1A) or as an accumulation ratio of tissue concentration to final medium concentration (C_t/C_m) (Figure 1B), was observed for promazine and amitriptyline. Drug uptake was reduced in the following order: promazine > amitriptyline > sertraline > perazine ≈ imipramine ≈ fluoxetine > thioridazine. Both the 'lysosomal inhibitors' ammonium chloride and monensin significantly decreased the drug uptake, except for promazine and fluoxetine, by vertically cut brain slices. The most pronounced effects of the 'lysosomal inhibitors', i.e., a 40–50% decrease in the absolute tissue accumulation (Figure 1A), were observed with thioridazine, perazine and sertraline.

The uptake of psychotropic drugs by slices of the grey and white matters of the brain

The results obtained show that the total uptake of the psychotropics by the grey matter (Figure 2A) of the brain was more pronounced than that by the white matter (Figure 2B), although the rank order of drug uptake by the grey and white matter was similar and as follows: sertraline > promazine ≈ imipramine ≈ amitriptyline ≈ fluoxetine > perazine > thioridazine. In contrast to the white matter, in the grey matter the

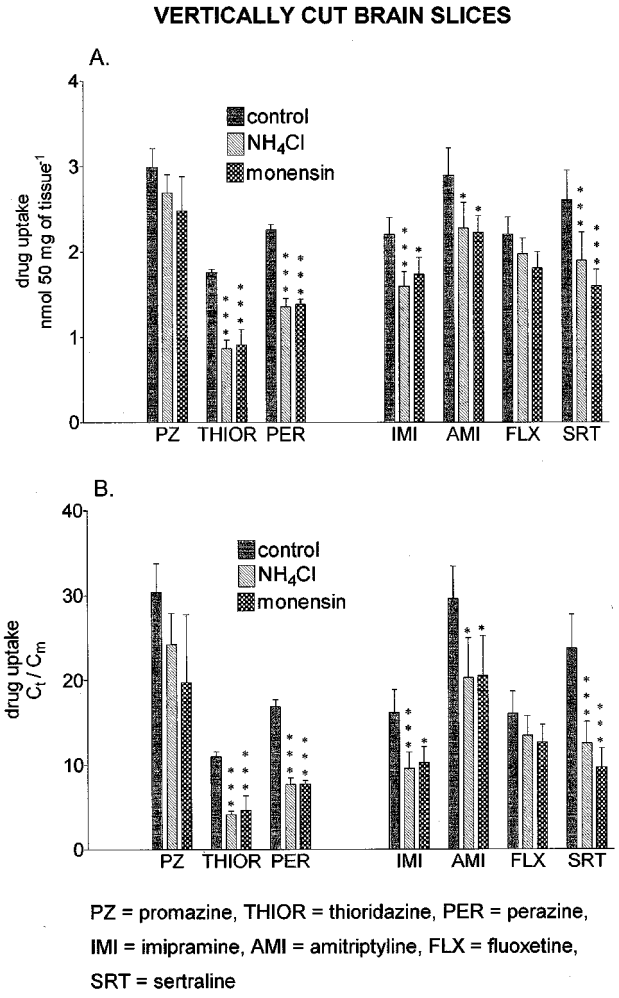

Figure 1 The influence of 'lysosomal inhibitors' on the tissue uptake of psychotropic drugs *in vitro*. Mean values ± s.d. ($n = 5-20$; n refers to an individual slice) are expressed as: (A) absolute tissue concentration, nmol 50 mg of tissue^{-1}; (B) an accumulation ratio of tissue concentration to final medium concentration, C_t/C_m. NH_4Cl (20 mM) or monensin (10 μM) was added to 1 ml of the Krebs-Henseleit buffer (pH = 7.4) containing 50 mg of tissue slices and one of the psychotropic drugs (5 μM). Incubation was carried out for 1 h. Statistical significance was assessed using Dunnett's test, and is indicated with $***P<0.001$, $*P<0.05$.

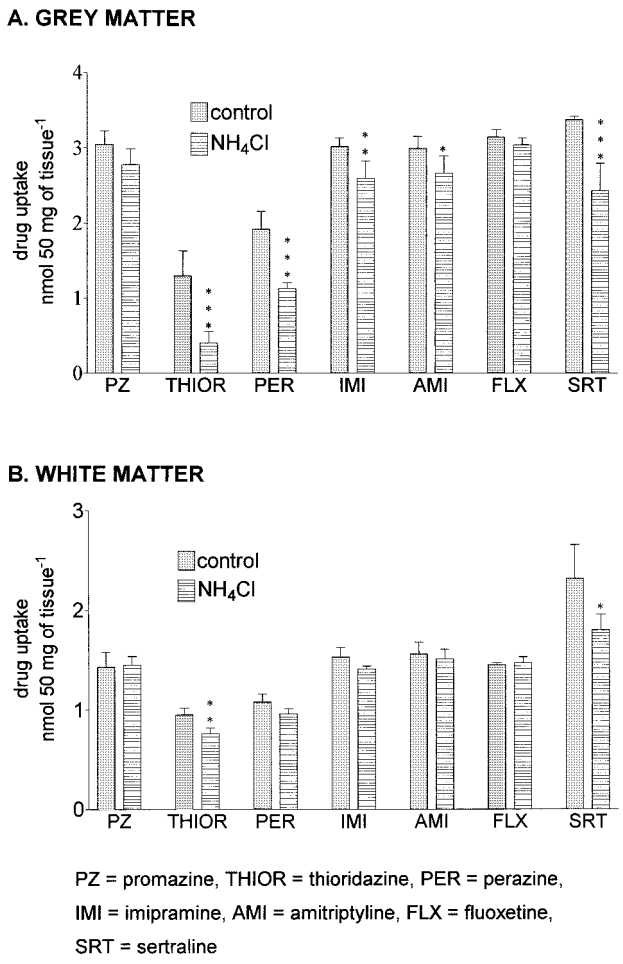

Figure 2 The uptake of psychotropic drugs by the grey and white matters of the brain, in the absence or presence of NH_4Cl. Mean values ± s.d. ($n = 5$; n refers to an individual slice) are presented. NH_4Cl (20 mM) was added to 1 ml of the Krebs-Henseleit buffer (pH = 7.4) containing 50 mg of tissue slices and one of the psychotropic drugs tested (5 μM). Incubation was carried out for 1 h. Statistical significance was assessed using Student's t-test, and is indicated with $***P<0.001$, $**P<0.01$, $*P<0.05$.

drugs showed distinct lysosomotropism, as shown by a decrease in the drug uptake in the presence of ammonium chloride. The exceptions were promazine and fluoxetine, the uptake of which by grey matter was not affected by ammonium chloride. Of the psychotropics studied, the most distinct lysosomotropic properties were shown by thioridazine and perazine, the uptake of which was reduced to 30 and 60% of control, respectively, in the presence of ammonium chloride (Figure 2A). The uptake of other psychotropics by the grey matter was approximately 70–90% of the control value in the presence of ammonium chloride. In the case of white matter (Figure 2B), only the uptake of thioridazine and sertraline was significantly decreased in the presence of the 'lysosomal inhibitor' but the observed decreases were not as large as in the case of grey matter (i.e. to 80% of the control value). For the majority of the psychotropic drugs tested, tissue binding (the uptake in the presence of ammonium chloride) was 1.5–2 times higher in grey matter than in white matter. The only exceptions were perazine (the binding of which to grey and white matter was similar) and thioridazine (which bound more potently to white matter) (Figure 2A,B).

The uptake of psychotropic drugs by neuronal and astroglial cell cultures

Total uptake of psychotropic drugs by neurones was 4–10 times greater than in astrocytes (Figure 3A,B). The highest neuronal uptake was observed for sertraline, thioridazine and imipramine (39.0–45.3 nmol mg protein^{-1}), and the highest astroglial uptake was reported for promazine and sertraline (7.5 nmol mg protein^{-1}). The lowest uptake into neurones was shown by fluoxetine, amitriptyline and promazine (27–30.5 nmol mg protein^{-1}), while the least uptake into astrocytes was noted with perazine and imipramine (3.3–3.7 nmol mg protein^{-1}). In contrast to astrocytes, all of the investigated psychotropic drugs displayed significant lysosomotropism in neurones, expressed as a decrease in their

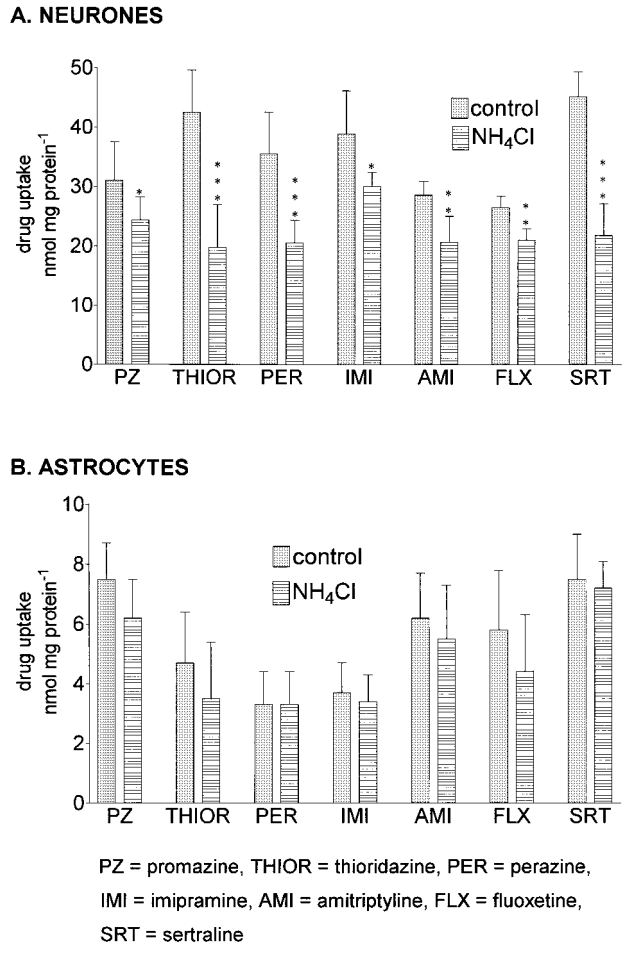

Figure 3 The uptake of psychotropic drugs by neuronal and astroglial cell cultures, in the absence or presence of NH_4Cl. Mean values ± s.d. ($n=5$; n refers to an individual culture well) are presented. NH_4Cl (20 mM) was added to 1 ml of the CGM containing neurons (0.04 mg protein ml^{-1}) or astrocytes (0.13 mg protein ml^{-1}) and one of the psychotropic drugs tested (5 μM). Incubation was carried out for 30 min. Statistical significance was assessed using Student's *t*-test, and is indicated with $***P<0.001$, $**P<0.01$, $*P<0.05$.

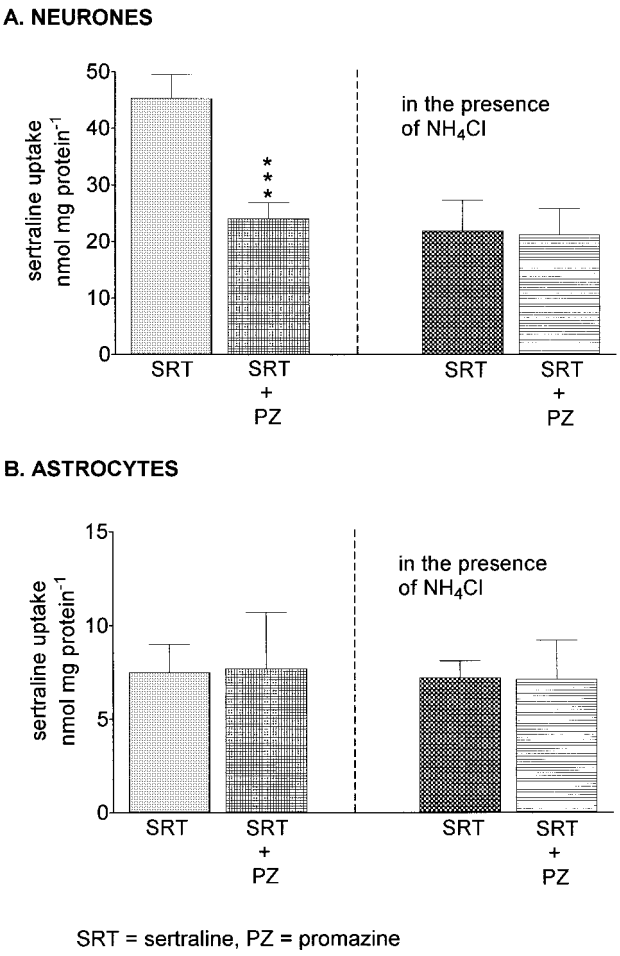

Figure 4 Sertraline-thioridazine interaction in the neuronal (A) and astroglial (B) cell cultures. The uptake of sertraline during its incubation alone or with promazine, in the absence or presence of NH_4Cl. Mean values ± s.d. ($n=5$; *n* refers to an individual culture well) are presented. NH_4Cl (20 mM) was added to 1 ml of the CGM containing neurons (0.04 mg protein ml^{-1}) or astrocytes (0.13 mg protein ml^{-1}) and sertraline (5 μM) or sertraline + promazine (5 μM each). Incubation was carried out for 30 min. Statistical significance was assessed using Dunnett's test, and is indicated with $***P<0.001$.

uptake in the presence of ammonium chloride. In neurones, the most pronounced lysosomotropic properties were exhibited by sertraline, thioridazine and perazine, the uptake of which was approximately 50% of the control value in the presence of ammonium chloride (Figure 3A). The uptake of other psychotropics by neurones reached 70–80% of the control value in the presence of the 'lysosomal inhibitor'. The uptake of the drugs by astrocytes was not affected by ammonium chloride (Figure 3B). The binding (the uptake in the presence of ammonium chloride) of the drugs to neurones (Figure 3A) was 3–8 times higher than to astrocytes (Figure 3B).

The first interaction experiment showed different effects of pharmacokinetic interaction between sertraline and promazine in neurones and astrocytes (Figure 4A, B). The distribution interaction between the two drugs took place only in neurones *via* inhibition of lysosomal trapping. Sertraline uptake was markedly diminished by its co-incubation with promazine in neuronal, but not astroglial cultures. A decrease in the neuronal uptake of sertraline was observed only in the absence of the lysosomotropic compound ammonium chloride, i.e. when the function of lysosomes was not disturbed. The second interaction experiment showed that thioridazine and imipramine mutually inhibited their uptake in neuronal cultures in the absence of ammonium chloride (Figure 5A, B), although in our previous experiment with vertically cut slices no effect of thioridazine on the uptake of imipramine was observed. Imipramine produced more potent inhibition of thioridazine uptake (a 66% decrease in thioridazine uptake) than did thioridazine to the uptake of imipramine (a 23% decrease in imipramine uptake).

Discussion

The results obtained with vertically cut brain slices showed that the total brain uptake of thioridazine, perazine, imipramine, amitriptyline and sertraline depends on both phospholipid binding (the uptake in the presence of

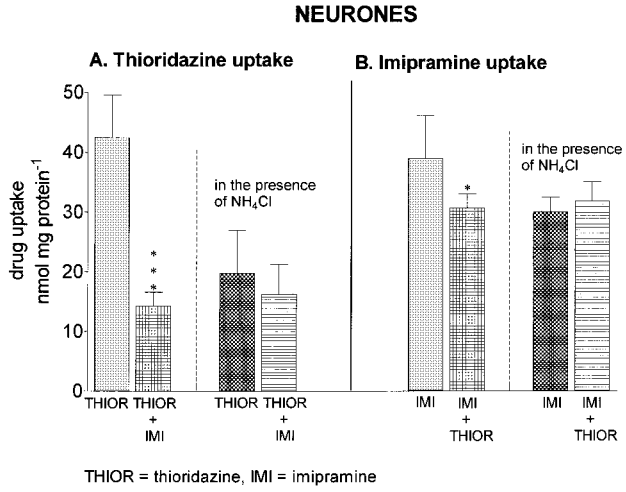

Figure 5 The thioridazine-imipramine interaction in the neuronal cell culture. The uptake of thioridazine (A) and imipramine (B) during separate or combined incubation, in the absence or presence of NH_4Cl. Mean values ± s.d. ($n = 5$; n refers to an individual culture well) are presented. NH_4Cl (20 mM) was added to 1 ml of the CGM containing neurons (0.04 mg protein ml^{-1}), thioridazine (5 μM) and/or imipramine (5 μM). Incubation was carried out for 30 min. Statistical significance was assessed using Dunnett's test, and is indicated with $***P<0.001$, $*P<0.05$.

ammonium chloride or monensin) and lysosomal trapping (the total uptake minus phospholipid binding), while the total uptake of promazine and fluoxetine apparently does not depend on lysosomal trapping. However, various types of cells in the brain are characterized by different lysosomal density and diverse lipid content and composition. Thus both the total uptake of drugs and the relative contribution of lysosomal trapping and tissue binding depend upon the brain area.

In our experiments, depending on the drug tested, total uptake by the grey matter was 1.5–2 times higher than uptake by white matter, and that by neurones was 4–10 times greater than by astrocytes. The 'lysosomal inhibitor' ammonium chloride decreased the uptake of the majority of psychotropic drugs by the grey matter (except for promazine and fluoxetine) while, in the case of the white matter, the uptake of thioridazine and sertraline was diminished only in the presence of ammonium chloride. Unlike in the vertically cut brain slices and grey matter, the uptake of all of the investigated drugs was reduced by ammonium chloride in a neuronal culture, but was unchanged in an astroglial culture. These findings indicate that lysosomal trapping plays an important role in the distribution of the psychotropics in neurones but not in glial cells. According to Kornhuber *et al.* (1995), slow accumulation of psychotropic drugs in acidic subcellular compartments of the brain (lysosomes), which alters a number of biochemical processes (by increasing the intralysosomal pH), may partly explain the therapeutic latency of these drugs.

The present results are in agreement with data obtained by other authors who found the highest density of lysosomes in neurones and such neuronal areas of the brain as the brain cortex, hypothalamus and basal ganglia (Sellinger & Hiatt, 1968; Sellinger & Nordrum, 1969; Idoyaga-Vargas *et al.*, 1972; Roberts & Gorenstein, 1987). Our results also correspond well with those of other authors who observed a higher activity of lysosomal enzymes in a mixed neuronal-glial culture than in astrocytes (Mandel *et al.*, 1978), and reported a higher activity of those enzymes in a neuronal fraction than in a fraction containing oligodendrocytes or astrocytes (Freysz *et al.*, 1979). The brain regions examined in our study, i.e. the brain cortex, representative of grey matter, and the corpus callosum and internal capsule, representative of white matter, have different proportions of neuronal and glial cells. The grey matter of the brain contains mainly neurones and only small numbers of glial cells (mainly astrocytes), while white matter abounds in oligodendrocytes and to a lesser extent in astrocytes, although axons and interneurons are also present there. Such differential cell composition of grey and white matter is reflected in a considerably higher lysosomal trapping of the psychotropics in grey matter of the brain. The above cell composition of the two brain matters also explains a slightly higher lysosomal trapping of these drugs in neuronal cultures compared with grey matter, as well as the lack of lysosomotropism of thioridazine and sertraline in astrocyte cultures compared to their weak lysosomotropism in the white matter. Therefore a less pronounced drug lysosomotropism and interactions involving this process cannot be observed when the experiment is carried out on vertically cut brain slices.

Our results indicate that in grey matter and neurones both lysosomal trapping and phospholipid binding are important for the cellular distribution and total uptake of these drugs, while in white matter and astrocytes binding to cellular elements prevails. Accordingly, the distribution interactions between antidepressants and neuroleptics at the level of lysosomal trapping (in the absence of ammonium chloride) take place in neurones, but not in astrocytes. Promazine decreased the uptake of sertraline in neurones but not in astrocytes. The interaction was observed only in the absence of the 'lysosomal inhibitor' which indicates that it occurred at the level of lysosomal trapping. Thioridazine and imipramine mutually inhibited their lysosomal trapping in neurones. However, in an earlier experiment with vertically cut brain slices, this effect of thioridazine on the distribution of imipramine was not observed, as it was masked by the presence of glial cells (Daniel & Wójcikowski, 1999b). A reduction (due to a distribution interaction) in the concentration of psychotropics in lysosomes (depot) may lead to an increase in their level in membranes and extralysosomal tissue fluids, i.e., at the site of their pharmacological action.

The observed higher total uptake of psychotropics by neurones than by astrocytes and by grey than by white matter is due not only to a varying degree of lysosomal trapping but also to differences in the binding to cellular elements. Surprisingly, the binding of the majority of psychotropics investigated in grey matter and neurones is higher than in white matter and astrocytes, although white matter contains almost twice as many lipids and proteins as grey matter (Söderberg *et al.*, 1990; Chavko *et al.*, 1993). Therefore it seems that in neurones there may exist some phospholipids and/or proteins with high affinity for these drugs. Specific neurotransmitter receptors (which bind neuroleptics and/or antidepressants) and neurotransmitter transporters involved in the regulation of biogenic amine uptake (which bind antidepressants) belong to these high-affinity binding sites, however, they seem to be only partly

responsible for the observed differences in binding, since their density is too low and they are saturated at nanomolar concentrations of the ligands. Possible candidates for psychotropic binders may be the negatively charged gangliosides, the concentration of which in grey matter is about 2.5 times higher than in white matter (Svennerholm & Vanier, 1972). According to the studies of Lüllman & Wehling (1979) the binding of drugs to different polar lipids may vary depending on the physicochemical properties of a drug and lipid. The binding of cationic drugs to anionic lipids may be higher than to such zwitterionic polar lipids as phosphatidylcholine, phosphatidylethanolamine or sphingomyelin. Moreover, differences in the fatty acid composition of the grey and white matter may also be of importance for the binding of the investigated psychotropic drugs (Skinner *et al*., 1993).

In summary, our study indicates that: (1) both lysosomal trapping and binding to cellular elements by psychotropics are higher in the grey matter and neurones than in the white matter and astrocytes, respectively, (2) the lysosomal trapping of psychotropics takes place mainly in neurones, (3) of the drugs investigated, thioridazine, sertraline and perazine show the highest degree of lysosomotropism in neurones, (4) distribution interactions between antidepressants and neuroleptics take place mainly in neurones at the level of lysosomal trapping, (5) a differential number of neuronal and glial cells in the brain may mask lysosomal trapping and distribution interactions of some less potent lysosomotropic drugs in vertically cut brain slices, (6) a decrease (*via* a pharmacokinetic interaction) in the concentration of psychotropics in lysosomes (depot) may lead to an increase in their level in membranes and tissue fluids (i.e. in concentrations relevant to their pharmacological action) and, in consequence, to enhancement of the drug binding to neurotransmitter receptors and/or transporters, (7) the interactions described may be of clinical importance, since antidepressants and neuroleptics are used jointly in the treatment of complex or 'resistant' psychiatric illnesses.

This study was supported by the Grant No. 4 PO5F 037 14 obtained from the Committee for Scientific Research (KBN), Warsaw. Thanks are due to Mrs Jadwiga Drabik for her excellent technical assistance.

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(Received March 14, 2001
Revised July 31, 2001
Accepted August 3, 2001)