In contrast to the serum CHE activity and albumin concentration (and possibly globulin concentration), the GPT and GOT activities in serum remained within the control levels throughout the experiment (data not shown).

The present study revealed that cadmium depresses the serum levels of not only CHE but also other secretory proteins from the liver, and this inhibitory action is observed before the onset of serum increase of leaked enzymes from the liver.

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Non-selective inhibition of GABA and 5-HT uptake systems in rat brain by *N*-*n*-alkyl hydroxybenzylamine and *N*-*n*-alkyl phenylethylamine derivatives

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GABA (4-aminobutyric acid) is believed to be the major inhibitory neurotransmitter used by the mammalian central nervous system (CNS) [1]. Accordingly, drugs which impair the inhibitory effects of GABA, by blocking its synthesis, release or post-synaptic actions, possess proconvulsant activity, whereas agents which enhance GABA mediated inhibition often exhibit anticonvulsant effects [2]. One approach to enhancing the inhibitory effects of synaptically released GABA is to block the high affinity, sodium dependent uptake process which transports GABA into GABAergic neurones and glial cells [3, 4]. When these GABA uptake mechanisms are inhibited, both the inhibitory effects of iontophoretically applied GABA, and physiologically evoked GABA mediated postsynaptic inhibition have been shown to be enhanced [5, 6]. Likewise, the central administration of GABA uptake inhibitors has been shown to protect genetically susceptible mice against sound-induced seizures [7]. Thus, it has been suggested that a potent and selective inhibitor of central GABA uptake mechanisms could be beneficial in the treatment of various neurological disorders, including epilepsy [2].

However, in addition to blocking GABA uptake, such a drug must also be able to cross the blood brain barrier. We have previously synthesized a series of *N*-*n*-alkyl hydroxybenzylamine derivatives (5–7) which inhibit the uptake of [^{3}H]GABA into rat brain synaptosomes [8]. Furthermore, the presence of long, hydrophobic, alkyl side chains on these molecules might be expected to facilitate their entry into the CNS. Sulphation of (6) on oxygen afforded a very potent GABA uptake inhibitor (IC_{50} 3.5 μm) which we regarded as a major lead because of its lipophilicity. This compound was, however, somewhat unstable to hydrolysis. With the apparent ability of the *n*-octyl chain to mask a zwitterion, it was clearly important to establish the generality of this structural device for the design of neurotransmitter analogues. In the present study, a number of *N*-octyl phenylethylamines (9–11) were also tested as inhibitors of [^{3}H]GABA uptake into crude P2 fractions prepared from rat cerebral cortex. Furthermore, to investigate their pharmacological specificity, the above compounds were examined for their ability to inhibit [^{3}H]5-HT uptake into crude P2 fractions prepared from rat hypothalamus.

Materials and methods

Chemical syntheses. Hydroxybenzylamines (5–7) were samples prepared and used in our previous studies [8, 9]. *N-octyl-2-methylbenzylamine* (8) was prepared by the method of Dewar *et al.* [9]; the hydrochloride had m.p. 169°. *Found:* C,71.1; H, 10.3; N, 5.0; Cl, 13.4; $C_{16}H_{28}ClN$ requires: C, 71.2; H, 10.5; N, 5.2; Cl, 13.1%. δ_H ($CDCl_3$) 7.8–7.1, 4H, m; 4.02, 2H, s; 2.43, ^{3}H, s; 2.8, 2H, m; 2.0–0.7, 15H.

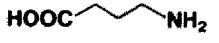
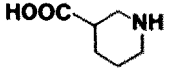
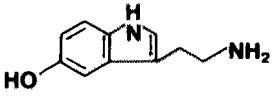
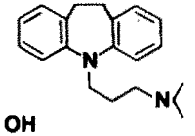
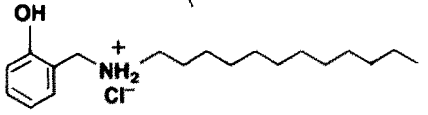
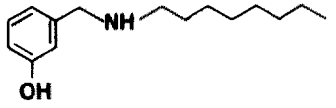
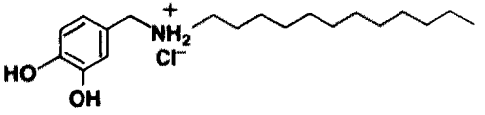
The *N-octylphenylethylamines* (9–11) were prepared by borane reduction of the corresponding amides which were obtained from the acid chlorides. *N-octyl-3,4-dimethoxyphenylacetamide* (93%) b.p. 112–114°, 0.1 Torr ν_{max} (Nujol): 3320, 1640, 1605, 780 and 720 cm^{-1}. δ_H ($CDCl_3$): 0.7–1.7, 15H, m; 3.2, 2H, *m*; 3.51, 2H, s; 3.88, 6H, s; 6.83, ^{3}H, s; 5.65, D_2O, b. *Found:* C, 70.1; H, 9.4; N, 4.2%. $C_{18}H_{29}NO_3$ requires C, 70.3; H, 9.5; N, 4.6%.

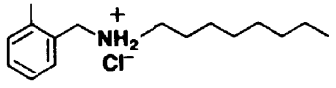
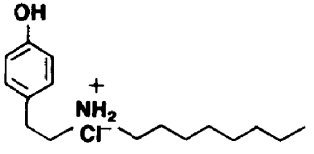
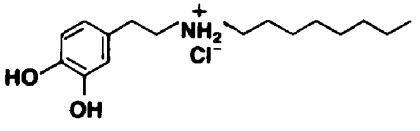
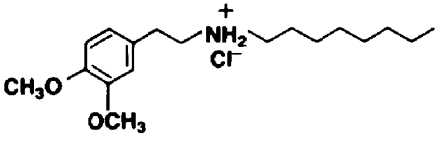
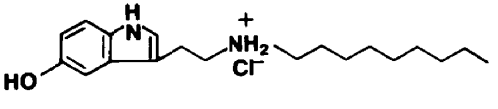
N-octyl-3,4-dimethoxyphenylethylamine hydrochloride (11) (61%) m.p. 116–118° ν_{max} (Nujol): 3200–2300, 1590, 800 and 720 cm^{-1}. δ_H ($CDCl_3$): 0.7–2.1, 15H, m; 2.96, 2H, m; 3.20, 2H, s; 3.86, 6H, s; 6.77, ^{3}H, s. *Found:* C, 65.1; H, 9.8; N, 4.4; Cl, 10.5%. $C_{18}H_{32}ClNO_3$ requires C, 65.6; H, 9.7; N, 4.3, Cl, 10.8%.

N-octyl-3,4-dihydroxyphenylethylamine hydrobromide (10) was prepared by demethylation of (11) using boron tribromide (63%) m.p. 108°. ν_{max} (Nujol) 3500–2500, 1600, 800 and 720 cm^{-1}. δ^H ($DMSOd_6$): 0.7–1.8, 15H, m; 2.88, 2H, m; 3.37, 2H, s; 6.4–6.8, ^{3}H, m; 8.4–8.9, b, D_2O. *Found:* C, 54.3; H, 8.1; N, 3.9; Br, 24.0%. $C_{16}H_{28}BrNO_2$ requires C, 55.5; H, 8.2; N, 4.1; Br, 23.1%.

N-octyl-4-hydroxyphenylacetamide (73%) m.p. 142–143° δ_H ($CDCl_3$): 0.7–1.7, 15H, m; 3.17, 2H, m; 3.44, 2H, s;

Table 1. IC_{50} values for the inhibition of [^{3}H]GABA uptake and [^{3}H]5-HT uptake into crude P2 fractions prepared from rat brain by various compounds

Compound	Structure	IC_{50} value (μM) [^{3}H]GABA uptake	IC_{50} value (μM) [^{3}H]5-HT uptake
1. GABA		7.0 ± 1.3	>1000
2. Nipecotic acid		4.6 ± 0.6	>1000
3. 5-HT		>1000	0.33 ± 0.04
4. Imipramine		23 ± 2.4	0.86 ± 0.06
5. *N*-dodecyl-2-hydroxybenzylamine		42 ± 0.3	43 ± 6.1
6. *N*-octyl-3-hydroxybenzylamine		33 ± 4.6	11 ± 3.1
7. *N*-dodecyl-3,4-dihydroxybenzylamine		33 ± 3.2	4.8 ± 2.5

8. *N*-octyl-2-methylbenylamine		75 ± 5.6	12.8 ± 1.8
9. *N*-octyl-4-hydroxyphenylethylamine		48 ± 3.1	14.7 ± 1.7
10. *N*-octyl-3,4-dihydroxyphenylethylamine		29 ± 2.2	6.9 ± 1.2
11. *N*-octyl-3,4-dimethoxyphenylethylamine		55 ± 2.9	2.8 ± 0.8
12. *N*-nonyl-5-HT		28 ± 5.8	4.1 ± 0.5

[^{3}H]GABA and [^{3}H]5-HT uptake were examined in crude P2 fractions of rat cerebral cortex and rat hypothalamus, respectively. IC_{50} values ± S.E.M. were determined as described in Materials and methods.

5.8, b, D_2O; 6.7–7.5, 4H, m. *Found:* C, 72.6; H, 9.4; N, 5.4%. $C_{16}H_{25}NO_2$ requires C, 73.0; H, 9.6; N, 5.3%.

N-octyl-4-hydroxyphenylethylamine hydrochloride (9) (39%) m.p. 152° ν^{max} (Nujol): 3500–2500, 1610 and 720 cm^{-1}. δ^H ($DMSOd_6$): 0.7–2.0, 15H; 2.7–3.1, 6H, m; 7.1, 4H, d.d. *Found:* C, 67.4; H, 10.0; N, 4.7; Cl, 12.5%. $C_{16}H_{28}ClNO_2$ requires C, 67.2; H, 9.9; N, 4.9; Cl, 12.4%.

N-nonyl-5-hydroxytryptamine (12) was prepared in protected form using the Reissert indole synthesis from 5-benzyloxy-2-nitrophenylpyruvic acid [10–12]. The product had m.p. 187° δ_H ($DMSOd_6$): 0.7–1.8, 17H, m; 2.80, 2H, t; 3.1, 2H, s; 5.15, 2H, s; 6.8–7.5, 4H, m; 8.0–9.0, b. *Found:* C, 67.4; H, 9.3; N, 8.4%. $C_{19}H_{31}ClN_2O$ requires C, 67.3; H, 9.2; N, 8.3%.

[^{3}H]*GABA uptake.* The uptake of [^{3}H]GABA into crude P_2 fractions prepared from rat cerebral cortex was measured as described previously [8] with slight modifications. Rat cerebral cortex was dissected and homogenised in 20 vol. of ice-cold 0.32 M sucrose, and centrifuged for 10 min at 1000 *g* and 4°. Fifty microlitre aliquots of the resultant supernatant (containing 0.2–0.3 mg protein) were added to incubation tubes containing 1 ml Krebs bicarbonate buffer (pH 7.4), together with either 10 μl of Krebs bicarbonate (to determine total uptake), or 10 μl of 100 mM GABA giving a final concentration of 1 mM (to determine 100% inhibition of [^{3}H]GABA uptake), or 10 μl of test drug.

Following a preincubation at 25° for 10 min, 10 μl of [^{3}H]-GABA (~1 nM [^{3}H]GABA in 0.1 μM unlabelled GABA) were added to each tube. After further 10 min incubation at 25°, the contents of each tube were filtered under vacuum through Whatman GF/B filters and washed once with 5 ml Krebs bicarbonate buffer (pH 7.4) at 25°. Filters were transferred to counting vials and radioactivity determined by scintillation counting. Specific [^{3}H]GABA uptake was determined by subtracting the radioactivity measured in the presence of 1 mM unlabelled GABA (blank) from the total [^{3}H]GABA uptake as determined in the absence of unlabelled GABA. Routinely, blanks represented approximately 5% of total [^{3}H]GABA uptake.

[^{3}H]*5-HT uptake.* The uptake of [^{3}H]5-HT into crude P_2 fractions prepared from rat hypothalamus was performed using a similar methodology to that described above. Rat hypothalami were dissected and homogenised in 10 vol. of ice-cold 0.32 M sucrose, and centrifuged for 10 min at 1000 *g* and 4°. Fifty microlitre aliquots of the resultant supernatant (containing 0.45–0.55 mg protein) were added to incubation tubes containing 1 ml Krebs bicarbonate (pH 7.4), modified by the addition of ascorbic acid (20 mg/l), EDTA (10 mg/l) and pargyline (19.6 mg/l), together with either 10 μl of Krebs bicarbonate (to determine total uptake) or 10 μl of 100 mM 5-HT giving a final concentration of 1 mM (to determine 100% inhibition of [^{3}H]-5-HT uptake), or 10 μl of test drug. Tubes were preincubated at 37° for 10 min prior to the addition of 10 μl of [^{3}H]5-HT (~6 nM [^{3}H]5-HT in 0.1 μM unlabelled 5-HT).

Following a further 10 min incubation at 37°, the contents of each tube were filtered under vacuum through Whatman GF/B filters and washed once with 5 ml Krebs bicarbonate buffer (pH 7.4) at 37°. Radioactivity associated with the filters was determined by liquid scintillation counting.

Specific [^{3}H]5-HT uptake was determined by subtracting the radioactivity measured in the presence of 1 mM unlabelled 5-HT (blank) from the total [^{3}H]5-HT uptake as determined in the absence of unlabelled 5-HT. Blanks represented approximately 20% of total [^{3}H]5-HT uptake.

Calculation of data. Test drugs were examined over the concentration range 10^{-8} to 10^{-3} M and data expressed as percentage inhibition of specific uptake. IC_{50} values (concentration of test drug producing 50% inhibition of specific uptake) were determined from the resultant inhibition curves. Within an experiment, each drug concentration was tested in triplicate. IC_{50} values for each test drug were determined from separate inhibition curves obtained from 3–6 experiments. Final data are given as the mean IC_{50} value ± S.E.M.

Materials. [^{3}H]GABA (54 Ci/mmol) and [^{3}H]5-HT (15.1 Ci/mmol) were purchased from the Radiochemical Centre (Amersham, U.K.).

Results and discussion

As illustrated in Fig. 1A, both GABA itself and nipecotic acid (2), a previously identified inhibitor of GABA uptake systems (13), potently inhibited the uptake of [^{3}H]GABA into crude P2 fractions of rat cerebral cortex with IC_{50} values of 7.0 μM and 4.6 μM, respectively (see Table 1). In marked contrast, 5-HT (IC_{50} > 1000 μM) was without effect on [^{3}H]-GABA uptake, with the classical 5-HT uptake blocker imipramine (4) (IC_{50} = 23 μM) being less active as compared with GABA or nipecotic acid (Fig. 1A; Table 1). On the other hand, the results shown in Fig. 1B indicate that whereas [^{3}H]5-HT uptake into crude P2 fractions of rat hypothalamus was potently inhibited by both 5-HT itself (IC_{50} = 0.33 μM) and imipramine (IC_{50} = 0.86 μM), GABA and nipecotic acid were inactive possessing IC_{50} values of >1000 μM (see Fig. 1B; Table 1). These data, therefore, confirm the pharmacological specificity of these two uptake systems. However, when tested as inhibitors of either [^{3}H]-GABA or [^{3}H]5-HT uptake, all of the various *N-n*-alkyl

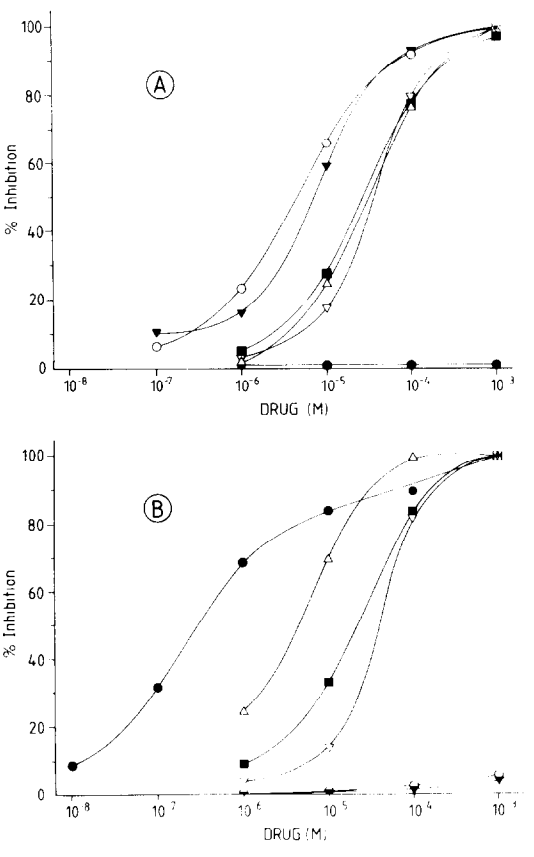


Fig. 1. Inhibition of (A) [^{3}H]GABA uptake into crude P2 fractions of rat cerebral cortex and (B) [^{3}H]5-HT uptake into crude P2 fractions of rat hypothalamus by GABA (▽), nipecotic acid (○), 5-HT (●). *N*-nonyl 5-HT (12) (△), *N*-octyl-3-hydroxybenzylamine (6) (□) and *N*-dodecyl-2-hydroxybenzylamine (5) (▽). Results were calculated as described in Materials and Methods. Each point is the mean of 3–6 separate experiments involving triplicate determinations at each drug concentration. S.E.M. values were less than 15% of the mean.

hydroxybenzylamine or *N-n*-alkyl phenylethylamine derivatives were moderately active as inhibitors of both uptake systems, possessing IC_{50} values of between 29 μM and 75 μM against [^{3}H]GABA uptake, and between 2.8 μM and 4.3 μM against [^{3}H]5-HT uptake (Fig. 1; Table 1).

It is probable that the ability of these *N-n*-alkyl derivatives to interact with both [^{3}H]GABA and [^{3}H]5-HT uptake systems relates to the surfactant-like properties of these highly lipophilic compounds and reflects a non-specific interaction with neuronal cell membranes. Evidence to support this suggestion comes from the fact that whereas 5-HT itself is a potent inhibitor of [^{3}H]5-HT uptake while being inactive against [^{3}H]GABA uptake, the *N-n*-nonyl (12) derivative of 5-HT inhibited both uptake systems (Table 1).

In summary, our results indicate that in addition to their ability to inhibit [^{3}H]GABA uptake in the brain, a series of *N-n*-alkylbenzylamine and *N-n*-alkylphenylethylamine derivatives were also moderately active at inhibiting central [^{3}H]5-HT uptake mechanisms. These results extend the significance of our initial observations concerning the O-sulphate of (6). Although low degrees of selectivity were observed with some compounds (7, 8, 11), these differences are too small to encourage the search for selectively acting drugs by this strategy. Thus attempts to improve the CNS penetrability of specific inhibitors of neurotransmitter uptake processes through the addition of *N-n*-alkyl side chains may result in the formation of non-specific inhibitors of CNS uptake processes.

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Regulation of cerebrovascular γ-glutamyltranspeptidase by adrenergic and cholinergic agonists

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Cerebral microvascular endothelium, the constituent cell of the blood–brain barrier, is enriched in the enzyme γ-glutamyltranspeptidase [(5-glutamyl)-peptide:amino acid 5-glutamyltranspeptidase, EC 2.3.2.2] [1, 2]. Elevated levels of this enzyme are found in several other cell types where amino acid transport is a prominent feature [3, 4]. Additionally, uptake of amino acids has been correlated with the level of transpeptidase activity [5]. These data suggest that γ-glutamyltranspeptidase plays a role in the regulation of amino acid uptake and transport. The factors that may modulate enzyme activity are unknown, though the demonstration of glial cell induction of cerebral endothelial transpeptidase indicates the importance of external factors [1]. Recent studies have demonstrated the presence of muscarinic cholinergic receptors on cerebral endothelial cells [6] and have shown that muscarinic and β-adrenergic receptors interact to determine the level of endothelial cAMP, a purported mediator of cerebrovascular permeability [7]. It has been also postulated that acetylcholine plays a role in amino acid transport [8] in non-nervous tissue. In this report, we test the hypothesis that adrenergic and/or cholinergic agonists, either independently or jointly, modulate the activity of γ-glutamyltranspeptidase in cerebral endothelium.

Methods

A microvessel preparation consisting primarily of capillary segments was isolated from the cerebral cortices of groups of twenty-four male Sprague–Dawley rats, as previously described [9]. The final microvessel pellet was resuspended in Dulbecco's modified essential medium containing 10% fetal calf serum supplemented with 20% dimethyl sulfoxide and stored in liquid nitrogen until use. Microvessels were quick-thawed and resuspended in phosphate-buffered saline (1 mM $CaCl_2$, 1 mM $MgCl_2$, 140 mM NaCl, 10 mM NaH_2PO_4, pH 7.4) (PBS). An alternate buffer, Hanks' balanced salt solution (137 mM NaCl, 5 mM KCl, 0.4 mM KH_2PO_4, 4 mM $NaHCO_3$, 0.6 mM Na_2HPO_4 6 mM glucose, pH 7.4 (HBSS), initially selected, yielded basal enzyme levels 60% lower than those obtained in PBS. Although the mechanism whereby incubation in PBS results in higher enzyme activity is unknown, the use of