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Distribution of [^{3}H]GR65630 Binding in Human Brain Postmortem

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We investigated the distribution of serotonin (5-HT) receptors of type 3 (5-HT$_3$) in human brain areas, by means of the the specific binding of [^{3}H]GR65630. The brains were obtained during autoptic sessions from 6 subjects. Human brain membranes and the binding of [^{3}H]GR65630 were carried out according to standardized methods. The highest density (Bmax ± SD, fmol/mg protein) of [^{3}H]GR65630 binding sites was found in area postrema (13.1 ± 9.7), followed at a statistically lower level, by nucleus tractus solitarius (6.7 ± 3.4), nervus vagus (5.5 ± 2.1), striatum (4.8 ± 2.4) with a progressive decrease in amygdala, olivar nuclei, hippocampus, olfactory bulbus and prefrontal cortex, and then by the other cortical areas and the cerebellum, where no binding was detected. These observations extend previous findings on the distribution of 5-HT$_3$ receptors and confirm interspecies variations that might explain the heterogeneous properties of 5-HT$_3$ receptors in different animals.

KEY WORDS: Serotonin; serotonin receptors of type 3; human brain postmortem; [^{3}H]GR65630 binding.

INTRODUCTION

The different actions of serotonin (5-hydroxytryptamine, 5-HT) seem to be mediated by various receptors which have been grouped into 7 distinct pharmacological families, most of which include different subtypes: 5HT$_1$, 5HT$_2$, 5HT$_3$, 5HT$_4$, 5HT$_5$, 5HT$_6$ and 5HT$_7$ (1). Unlike the other 5-HT receptors which belong to the G-protein-coupled receptor superfamily, the 5-HT$_3$ subtype is represented by a pentameric membrane ligand-gated ion channel, permeable to Na^+, Ca^{2+} and K^+ ions whose influx provokes the cell depolarization (2). The 5-HT$_3$ receptors, originally called "M", are distributed in both peripheral tissues and brain areas. In periphery, they are mainly localized in the enteric nervous system and in the pre- and post-ganglionic neurons. In the brain, the highest density is found in the area postrema, followed by the nucleus tractus solitarius, trigeminal nucleus, dorsal vagal complex, substantia gelatinosa and, at a lower density, in the hippocampus, habenula, amygdala and various cortical areas (3–6).

The stimulation of these receptors produces vasodilation, inhibition or stimulation of the cardiac, intestinal and lung functions or pain, and induces nausea and vomiting. The development of selective 5-HT$_3$ receptor antagonists has provided very powerful compounds against emesis provoked by chemio- and radiotherapy (7). When given centrally, 5-HT$_3$ receptor antagonists display anxiolytic and antipsychotic activity in some, but not all animal models. For this reason, any attempt to transfer these observations into clinical practice has proven unsatisfactory until to now, although research in this field is in progress (8,9). These controversies may be explained by the existence of interspecies variations in 5-HT$_3$ receptors.

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We aimed to contribute to available information on 5-HT_3 receptors in the human brain in order to understand their possible role, by investigating their distribution in different human brain areas obtained postmortem, by means of the specific binding of [^{3}H]-GR65630, a potent and selective 5-HT_3 receptor antagonist.

EXPERIMENTAL PROCEDURE

Human Brain Tissue. Human brain tissue was obtained at autopsy at the Institute of Pathology, University of Pisa, from 6 different subjects (3 men, and 3 women, between 54 and 80 years of age, mean ± SD: 66.4 ± 12.4) who had died from causes which did not involve the central nervous system, either primarily or secondarily and whose histories excluded any evidence of psychiatric disorders or treatment with psychotropic drugs before death, kept separate and processed separately. Causes of death were heart failure (2), myocardial infarction (2) and respiratory failure (2). The postmortem delay ranged between 10 and 46 hours (mean ± SD: 26 ± 9).

The area postrema, nucleus tractus solitarius, nucleus nervus vagus, striatum, amygdala, olivar nuclei, hippocampus, olfactory bulbus, prefrontal, parietal, occipital and temporal cortex and cerebellum, were identified by the anatomists, then cut into blocks, rapidly frozen in liquid nitrogen and stored at −80°C until the assay, which was performed within 2 weeks.

This study was approved by the Ethics Committee of Pisa University.

Membrane Preparation. Human brain membranes were prepared as described by Marazziti et al. (10). Tissue blocks were weighed, homogenized by means of an ultraturrax T25 homogenizer at half-maximum speed for about 30 sec. In 10 volumes of ice-cold 0.32 M sucrose, containing protease inhibitors (200 μg/ml benzamidine, 160 μg/ml bacitracin and 20 μg/ml soybean trypsin inhibitor) and then centrifuged at 1000 *g* for 5 min at 4°C. The resulting pellet was discarded, whereas the supernatant was centrifuged at 49,000 *g* for 15 min at 4°C. The pellet was resuspended in 10 volumes (w/v) of ice-cold Tris HCl 50 mM, pH 7.4 with protease inhibitors and subsequently incubated being stirred at 37°C for 15 min to destroy endogenous 5-HT. The homogenate was then aliquoted and centrifuged as above. The final pellets were stored at −80°C until assay.

[^{3}H]GR65630 Binding Assay. The[^{3}H]GR65630 binding was carried out according to the method of Kilpatrick et al. (4). Hepes buffer 50 mM pH 7.4, was incubated in the presence of [^{3}H]GR6563 (specific activity: 65.8 Ci/mmol from NEN, Boston, MA U.S.A) 0.5 nM for 45 min at 25°C in a final volume of 1 ml. The non-specific binding was accounted for by incubating similar samples with 5 μM quipazine (Sigma). Saturation curves were carried out while incubating [^{3}H]GR65630 at 8 concentrations ranging from 0.01 to15 nM. The distribution of 5-HT_3 receptors was evaluated by incubating the [^{3}H]-GR65630 at saturation concentration (5 nM).

The incubation was halted by the addition of 5 ml of ice-cold buffer. Samples were rapidly vacuum-filtered through Whatman GF/C glass filters, 2.5 cm in diameter, and then washed twice with 5 ml of ice-cold T1 buffer. Filters were then placed in vials with 4 ml of Ready Protein scintillation cocktail (Beckman) and radioactivity was measured by means of a beta-counter (Packard 1600 Tricarb).

Proteins were measured according the Lowry's method modified by Peterson (11), using bovine serum albumin as the standard.

Data Analyses and Statistics. The equilibrium-saturation binding parameters, i.e. the maximum binding capacity (B_{max}, fmol/mg protein) and the dissociation constant (K_d, nM), were analysed by means of the iterative curve-fitting computer programme, EBDA-LIGAND (12), using an IBM-compatible personal computer. The difference in binding parameters amongst the various brain areas was measured according to analysis of variance.

RESULTS

As shown in Table I, the highest density of [^{3}H]GR65630 binding sites was found in the area postrema and then, at a statistically significant lower level, in the nucleus tractus solitarius, nervus vagus and striatum, while the K_d values were not significantly different.

The Scatchard analysis in these same areas revealed the presence of a single population of high-affinity binding sites, as the Hill number was close to unit (Fig. 1).

This analysis also revealed that the striatum may be considered the limit area for the sensitivity and reliability of the binding method. Therefore, for a comparison of the remaining areas, the receptor distribution was evaluated while considering K_d as a constant and while utilizing a radioligand concentration saturating all receptors: the specific binding obtained in this way was then divided according to mg of proteins. This

Table I. [^{3}H]-GR65630 Binding Parameters (B_{max} and K_d) in Some Human Brain Membranes

	Area postrema	Nucleus tractus solitarius	Nucleus nervus vagus	Striatum
B_{max} (fmol/mg protein)	13.1 ± 9.7	6.7 ± 3.4	5.5 ± 2.1	4.8 ± 2.4
K_d (nM)	0.6 ± 0.2	0.47 ± 0.28	0.5 ± 0.3	0.4 ± 0.3

B_{max} (fmol/mg protein) and K_d (nM) values are means ± SD from three experiments in triplicate.

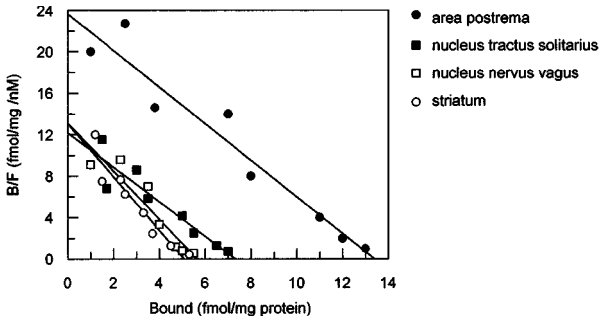

Fig. 1. Scatchard analysis of the specific binding of [^{3}H]GR65630 in human brain membranes from different areas. Results from a single experiment are presented where the total binding and the non-specific binding (defined by the presence of quipazine 5 μM) were determined in triplicate to allow calculation of the specific binding (data from one experiment are used to generate the mean values: Table I).

method, also, confirmed that the richest area in 5-HT$_3$ receptors is the area postrema, followed by the nucleus tractus solitarius, nervus vagus and striatum, with a progressive decrease in amygdala, olivar nuclei, hippocampus, olfactory bulbus and prefrontal cortex, until the other cortical areas and the cerebellum where no binding was detected (Fig. 2).

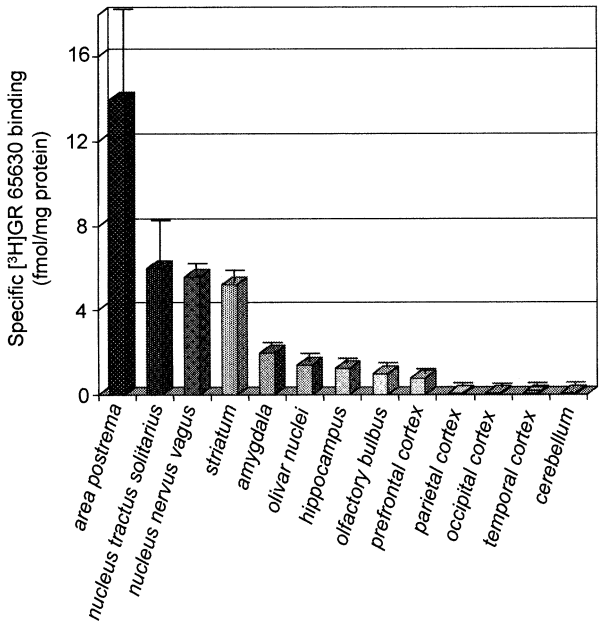

Fig. 2. Distribution of [^{3}H]GR65630 binding in different human brain areas. Level of [^{3}H]GR65630 (5 nM) labeled 5-HT$_3$ receptors in human brain (non-specific binding was defined by the presence of quipazine 5 μM). Data represent the means ± SEM.

DISCUSSION

The major findings of the present study are represented by data on the distribution of 5-HT$_3$ receptors in the human brain, by means of the binding of [^{3}H]GR65630, a specific antagonist at this level. We have analysed more brain areas than in the previous studies and we have also estimated the maximum densities, instead of utilizing a single ligand concentration (4) or autoradiography (13). The richest area in 5-HT$_3$ receptors was represented by the area postrema, followed by the nucleus tractus solitarius and nervus vagus and striatum. The distribution in the first 3 areas is consistent with previously reported high levels of 5-HT$_3$ receptors in human and animal (mouse, rat, ferret, cat) brainstem with different radioligands (3,14–17). It is widely agreed that the 5-HT$_3$ receptors in this region are involved in the regulation of emesis and that they represent the primary target of 5-HT$_3$ receptor antagonists used in chemio- and radiotherapy (7).

On the other hand, the high density found in the striatum would seem to be specific to the human brain (18), since in most animal species the levels of these receptors are below the limits of detection of the methods used (3,19). The functional significance of this localization is still unclear: however, a case report of a patient presenting extrapyramidal side-effects following ondansetron is interesting (20).

We measured significantly and progressively lower levels of 5-HT$_3$ receptors in different limbic areas, such as the amygdala, hippocampus and olfactory bulbus. This observation is consistent with previous data and suggests a possible role of 5-HT$_3$ receptors in some of the functions attributed to these regions, such as cognition and memory, anxiety and psychosis (8), as well as responses to alcohol and drugs of abuse (21,22); however, the transfer into clinical practice of data regarding the effectiveness of 5-HT$_3$ receptor antagonists obtained from animal models of the various disorders, has proved disappointing.

The prefrontal cortex was the last area were we could measure the [^{3}H]GR65630 binding, while no binding was detected in the other cortical areas and the cerebellum: this is at variance with animal distribution, where high levels are detected in cortical areas.

In conclusion, our study extends previous data on the distribution of 5-HT$_3$ receptors in the human brain and confirms interspecies variations in these receptors that might explain the difficulty in transferring animal data to clinical practice for various neuropsychiatric symptoms and disorders.

REFERENCES

1. Boess, F. G. and Martin, I. L. 1994. Molecular biology of 5-HT receptors. Neuropharmacology 33:275–317.
2. Hoyer, D. and Martin, G. 1997. 5-HT receptor classification and nomenclature: towards a harmonization with the human genome. Neuropharmacology 36:419–428.
3. Kilpatrick, G. J., Jones, B. J., and Tyers, M. B. 1987. Identification and distribution of 5-HT3 receptors in rat brain using radioligand binding. Nature 330:746–748.
4. Kilpatrick, G. J., Jones, B. J., and Tyers, M. B. 1989. Binding of the 5-HT_3 ligand [^{3}H]GR65630, to rat area postrema, vagus nerve, and the brains of several species, Eur. J. Pharmacol. 159: 157–164.
5. Flechter, S. and Barne, N. M. 1999. Autoradiographic localization of [^{3}H]-(S)-zacopride labelled 5-HT_3 receptor in bovine brain. Neurosci. Lett. 269:91–94.
6. Jones, D. N. C., Barnes, N. M. Costall, B., Domeney, A. M., Kilpatrick, G. J., Naylor, R. J., and Tyers, M. B. 1992. The distribution of 5-HT_3 receptor recognition sites in the marmoset brain. Eur. J. Pharmacol. 215:63–68.
7. Plosker, G. L. and Goa, K. L. 1991. Granisetron—a review of its pharmacological properties and therapeutic uses as an antiemetic. Drugs 42:805–824.
8. Bloom, F. E. and Morales, M. 1998. The central 5-HT_3 receptor in CNS disorders. Neurochem. Res. 23:653–659.
9. Barnes, J. M., Barnes, N. M., and Cooper, S. J. 1992. Behavioral pharmacology of 5-HT_3 receptors. Neurosci. Biobehav. Rev. 16:107–113.
10. Marazziti, D., Rossi, A., Palego, L., Giannaccini, G., Naccarato, A., Lucacchini, A., and Cassano, G. B. 1997. [^{3}H]Ketanserin binding in human brain postmortem. Neurochem. Res. 22: 753–757.
11. Peterson G. L. 1977. A semplification of the protein assay method of Lowry *et. al.*, which is more generally applicable. Anal. Biochem. 83:356–366.
12. Mc Pherson G. A. and Grant A., Kinetic, Ebda, Ligand, Lowry, A collection of radioligand binding analysis programs. Biosoft, Cambridge (1985).
13. Parker, R. M. C., Barnes, J. M., Ge, J., Barber, P. C., and Barnes N. M. 1996. Autoradiographic distribution of [^{3}H]-(S)-zacopride-labelled 5-HT_3 receptors in human brain. J. Neurol. Sci. 144: 119–127.
14. Pratt, G. D., Bowery, N. G., Kilpatrick, G. J., Leslie, R. A., Barnes, N. M., Naylor, R. J., Jones, B. J., Nelson, D. R., Palacios, J. M., Slater, P. and Reynolds, J. M. 1990. The distribution of 5-HT_3 receptors in mammalian hindbrain—a consensus. Trends Pharmacol. Sci. 11:135–137.
15. Waeber, C., Hoyer, D., and Palacios, J. M., 1989. 5-hydroxytryptamine3 receptors in the human brain: Autoradiographic visualization using [^{3}H]-ICS 209–930, Neuroscience 31:393–400.
16. Perry, D. C. 1990. Autoradiography of [^{3}H]-quipazine in rodent brain. Eur. J. Pharmacol. 187:75–80.
17. Gehlert, D. R., Gackenheimer, S. L., Wong, D. T. and Robertson, D. W. 1991. Localization of 5-HT3 receptors in the rat brain using [^{3}H]-LY278584. Brain Res. 553:149–154.
18. Abi-Dargham, A., Laruelle, M., Wong, D. T., Robertson, D. W., Weinberger, D. R., and Kleinman, J. E. 1993. Pharmacological and regional characterization of [^{3}H]LY278584 binding sites in human brain. J. Neurochem. 60:730–737.
19. Barnes, J. M., Barnes, N. M., Costall, B., Deakin, J. F., Ironside, J. W., Kilpatrick, G. J., Naylor, R. J., Rudd, J. A., Simpson, M. D., and Slater, P. 1990. Identification and distribution of 5-HT3 recognition sites within the human brain stem. Neurosci. Lett. 111:80–86.
20. Jacobsen, M. B. J. 1992. Ondansetron in carcinoid syndrome. Lancet 340:185.
21. Walsh, S. L. and Cunnigham, K. A. 1997. Serotonergic mechanisms involved in the discriminative stimulus, reinforcing and subjective effects of cocaine. Psychopharmacology 130:41–45.
22. Lovinger, D. M. 1999. 5-HT_3 receptors and the neural actions of alcohols: an increasingly exciting topic. Neurochem. Int. 35: 125–130.