5-HT1 receptors in the vertebrate brain
Regional distribution examined by autoradiography

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Summary. The regional distribution of high affinity [3H]5-HT recognition sites in the brain of several vertebrates (pigeon, rat, mouse, guinea-pig, cat, dog, monkey and human) was analyzed using in vitro autoradiography. The presence of subtypes of 5-HT1 binding sites was investigated by selective displacements with 8-OH-DPAT, mesulergine and (+)-SDZ 21-009 at appropriate concentrations to block 5-HT1A, 5-HT1C and 5-HT1B sites respectively. In addition, 5-HT1A and 5-HT1C sites were directly visualized with the more selective radioligands [3H]8-OH-DPAT and [3H]mesulergine, respectively. In the pigeon brain, total [3H]5-HT binding sites were enriched in all telencephalic areas. Densely labelled regions were also present in the optic tectum and the brainstem. No binding was observed in the cerebellum. 8-OH-DPAT and mesulergine only displaced a small proportion of [3H]5-HT binding in most of the areas where high concentrations of 5-HT1 sites were found. (+)-SDZ 21-009 did not affect [3H]5-HT binding in the regions examined. Taking into account our pharmacological studies, these results suggest that the majority of 5-HT1 sites belong to the 5-HT1D subtype in the pigeon brain. In the mammalian species investigated high levels of [3H]5-HT binding were found in the neo-cortex, hippocampal formation, basal ganglia and related structures (substantia nigra), raphe dorsalis, nucleus superior colliculus and choroid plexus. However, these brain areas were differentially enriched in subtypes of 5-HT1 recognition sites. 5-HT1A sites were observed in the neo-cortex, the hippocampal formation and the raphe nucleus, whereas 5-HT1C sites accounted for all 5-HT1 binding in the choroid plexus. In the mouse and rat brain, 5-HT1B binding sites were enriched in the basal ganglia and associated regions (substantia nigra). These areas were enriched in 5-HT1D sites in the brain of the other mammals studied. In these animals, no site with a 5-HT1B pharmacological profile were detected. These results indicate that 5-HT1A, 5-HT1C and 5-HT1D sites are present already in the lower vertebrate species investigated and that 5-HT1B appear to be exclusive of the myomorph rodents (mouse, rat). Furthermore, the different subtypes of the 5-HT1 receptors present a conserved regional distribution with the 5-HT1D sites being enriched in the basal ganglia and the 5-HT1A sites predominating in the hippocampal formation.

Key words: Serotonin - [3H]5-HT - 5-HT1B and 5-HT1D sites - Autoradiography - Species differences - Basal ganglia

Introduction
Serotonin (5-HT, 5-hydroxytryptamine) is found in neurons in the nervous system of even the most primitive species (Schwartz and Shkolnik 1981). Multiple receptors for 5-HT have been shown to be present already in the lower invertebrate species (Gerschenfeld and Paupardin-Tritsch 1974). In the mammalian brain, the existence of at least 8 different subtypes of 5-HT receptors has been proposed (Peroutka 1988). Species differences in the pharmacological characteristics of these receptor subtypes appear to exist (Schnellmann et al. 1984; Heuring et al. 1986; Hoyer et al. 1985, 1986). In our previous biochemical studies (Waeger et al. 1989), we have shown that the majority of [3H]5-HT binding sites in the pigeon brain are accounted for by sites with a 5-HT1D pharmacological profile. The brain of this vertebrate also contains sites labelled by [3H]8-OH-DPAT and [3H]mesulergine, while 5-HT1B recognition sites have not been demonstrated. 5-HT1 sites present a very heterogeneous distribution in the rat and human brain where different areas are enriched in one or the other subtype. We have used receptor autoradiography to examine the distribution of 5-HT1 receptors in the brain of several animal species which are commonly used to study the effects of serotoninergic compounds. In this paper, we focus particularly on 5-HT1B and 5-HT1D sites, 5-HT1A and 5-HT1C have been extensively described in rats and humans (Pazos and Palacios 1985; Pazos et al. 1987).

Our goal was to analyze possible species differences in the anatomical distribution of these sites, the relation of these differences with the evolution of the vertebrate brain and the identification of animal species which are closer to the situation found in the human brain, thus providing an appropriate animal model for the study of neurological diseases, involving in particular movement disorders.

Materials and methods
Human brains were obtained at autopsy from 8 subjects (3 males, 5 females, aged 72 ± 6 years, post-mortem delay 13 ± 4 h) deceased without any reported history of neurological or psychiatric disease. One centimeter thick tissue blocks were excised, frozen on dry ice and kept at −20°C.
Rhesus monkeys, cats and dogs were killed by an overdose of phenobarbital. Rats, guinea-pigs and mice were killed by decapitation. Animal brains were dissected, frozen on dry ice and stored at −20°C. 10 μm thick sections were cut from different brain areas or levels with a microtome-
cryostat, mounted on gelatin-coated slides and stored at -20°C until used.

Incubations were performed according to the following procedure: after a 30 min preincubation in 170 mmol/l Tris-HCl pH 7.4 containing 4 mmol/l CaCl₂ and 0.01% ascorbic acid, the slides were incubated for 1 hour at room temperature in the same medium supplemented with 10 mmol/l pargyline and 2 mmol/l [³H]5-HT (26.3 Ci/mmol). Non specific binding was determined by incubating consecutive sections in the presence of 10 μmol/l 5-HT. 100 nmol/l 8-OH-DPAT, 100 nmol/l mesulergine and 30 nmol/l (±)SDZ 21-009 were added in some conditions to displace [³H]5-HT from 5-HT₁₅, 5-HT₁₇ and 5-HT₁₈ sites, respectively. The washing process was carried out at 4°C as follows: a brief dipping in preincubation buffer followed by a 5 min wash in the same buffer and a brief dipping in distilled water to remove the salts. Finally, the sections were rapidly dried under a stream of cold air. Autoradiograms were generated by apposing the labelled tissues to ³H-Hyperfilms (Amersham, UK) along with Amersham tritiated plastic standard. Films were developed after 2 months exposure at 4°C. Autoradiograms were quantified using a computerized

Figures 1 to 5. Pictures showing the autoradiographic localization of [³H]5-HT binding sites in the brain of the guinea-pig, rat, mouse, cat, dog, man and pigeon. Tissues were incubated with 2 nmol/l [³H]5-HT alone or in the presence of compounds specific for different subtypes, as described in Materials and methods. Dark areas are regions with high receptor density. Non specific binding, shown in Fig. 1 E, is equal to film background and is representative of that observed in all regions examined.

Fig. 1. Coronal (A, B) and horizontal (C-E) sections of the pigeon brain. A and C represent total [³H]5-HT binding, B and D display binding remaining in the presence of 100 nmol/l 8-OH-DPAT and 100 nmol/l mesulergine. E is an illustration of the very low non specific binding obtained by incubation in the presence of 1 μmol/l 5-HT. Note overall high level of 5-HT₁ binding in the telencephalon (A, upper part of C). 5-HT₁₇ sites are enriched in the paleostriatum primitivum (PP), the accumens (Acc), the internal part of the superficial layer of the optic tectum (OT) and, to a lesser extent, in the paleostriatum augmentatum (PA), the lateral septum (LS), the ectostriatum (E) and the hyperstriatum ventrale (HV)

Fig. 2. Coronal sections of guinea-pig (A, D, G), rat (B, E, H) and mouse (C, F, I) brains, at the level of the nucleus lentiformis. A-C display total [³H]5-HT binding, D-F represent binding remaining in the presence of 100 nmol/l 8-OH-DPAT and 100 nmol/l mesulergine and G-I show binding after addition of 30 nmol/l (±)SDZ 21-009 to these 2 compounds. Note high level of non 5-HT₁₅ non 5-HT₁₇ binding (D-F) in the caudate-putamen (CPu) and the globus pallidus (GP) of all rodents, (±)SDZ 21-009 displaying an efficient displacement only in the rat and mouse brain. High densities of total 5-HT₁ binding are accounted for by 5-HT₁₅ sites in the lateral septum (LS) and the amygdaloid complex (A) of all rodents and in the claustrum (Ci) of the rat and mouse. The choroid plexus contains very high concentrations of 5-HT₁₇ binding sites. Bars are 25 mm