Abstract In the mammalian mesencephalon, virtually all serotoninergic neurons are located in the raphe nuclei and the adjacent reticular formation. Pharmacological evidence obtained in rodents suggests that terminal and somatodendritic autoreceptors controlling serotonin (5-hydroxytryptamine, 5-HT) release belong to the 5-HT$_{1B/D}$ subtype of receptors, whereas somatodendritic autoreceptors controlling neuronal cell firing are predominantly of the 5-HT$_{1A}$ subtype. This study investigated the presence of h5-HT$_{1D}$ and h5-HT$_{1B}$ receptor mRNA within the subdivisions of the dorsal raphe of post-mortem human brains by means of in situ hybridisation. Although differences in the labelling intensity, which may be caused by different pre- and/or post-mortem conditions, were obvious among the specimens, all brains expressed both the h5-HT$_{1D}$ and the h5-HT$_{1B}$ mRNA in dorsal raphe neurons. In comparison to h5-HT$_{1D}$ mRNA, expression of h5-HT$_{1B}$ mRNA was slightly more abundant. Information on the existence and localisation of h5-HT$_{1D}$ and h5-HT$_{1B}$ receptors in human dorsal raphe neurons confirms that both subtypes may serve an autoreceptor function in humans. This finding is of pharmacological relevance since these receptors are potential new targets for therapeutic interventions in psychiatric disorders such as depression and anxiety.

Keywords 5-HT$_{1B/D}$ serotonin receptors · Autoreceptors · Heteroreceptors · In situ hybridisation

Introduction Serotonergic neurons are mainly localised in the raphe nuclei and project to virtually all regions of the brain (Dahlström and Fuxe 1964; Wilson and Molliver 1991). In contrast to the ionotropic 5-HT$_3$ receptor actions, the G-protein-mediated responses to serotonin (5-hydroxytryptamine, 5-HT) are exerted via several receptors: 5-HT$_{1–2,4–7}$ and their subtypes (for review see Barnes and Sharp 1999). According to their localisation and actions they have been identified as postsynaptic receptors, as autoreceptors with electrophysiological functions or modulating the release of 5-HT and as presynaptic heteroreceptors modulating the release of other neurotransmitters like acetylcholine or noradrenaline (Barnes and Sharp 1999; Göthert and Schlicker 1997). 5-HT$_{1B/D}$ receptors (previously also designated as 5-HT$_{1D}$/$\alpha$/$\beta$; for new nomenclature see Hartig et al. 1996) have also been described as auto- as well as heteroreceptors (Barnes and Sharp 1999). In humans they share 77% sequence homology and both are negatively coupled to adenylate cyclase in second messenger cascades (Adham et al. 1992; Hamblin and Metcalf 1991; Weinshank et al. 1992). Furthermore, they have almost indistinguishable profiles of ligand binding exhibiting high affinity for GR 127 935 (N-[4-methoxy-3-(4-methyl-1-piperazinyl) phenyl]-2'-methyl-4’-(5-methyl-1,2,4-oxadiazol-3-yl) [1,1-biphenyl]-4-carboxamide) and GR 125 743 (N-[4-methoxy-3-(4-methyl-1-piperazinyl) phenyl]-3-methyl-4-(4-pyridinyl) benzamide; Pauwels 1997; Pauwels et al. 1996). Only the rat r5-HT$_{1B}$ receptor shows higher affinity for $\beta$-adrenoceptor ligands and 3-[1,2,5,6-tetrahydropyridin-4-yl] pyrrolo-[3,2-b] pyrid-5-one (CP 93 129) whereas a slightly lower affinity for sumatriptan has been described (Barnes and Sharp 1999). More selective receptor ligands like SB 216641 (N-[3-(2-dimethylamino) ethoxy-4-methoxyphenyl]-2'-...
methyl-4’-(5-methyl-1,2,4-oxadiazol-3-yl)-(1,1’-biphenyl)-4-carboxamide) for 5-HT<sub>1B</sub> Receptors and BRL 15572 (3-[4-(3-chlorophenyl) piperazin-1-yl]-1,1-diphenyl-2-propanol) for 5-HT<sub>1D</sub> receptors have become available only recently (Göttert and Schlicker 1997).

The cerebral distribution of 5-HT<sub>1D</sub> or 5-HT<sub>1B</sub> receptors has been described by mRNA expression or differential radioligand binding using in vitro receptor autoradiography in several species (Bruinvels et al. 1993; Del Arco et al. 1993; Neumaier et al. 1996) including the human forebrain (Bruinvels et al. 1994b; Pasqualetti et al. 1996). Only for the guinea-pig it has been shown that, despite their great differences within their cerebral expression patterns, both 5-HT<sub>1B</sub> and 5-HT<sub>1D</sub> receptors are highly expressed in dorsal raphe nuclei (Bonaventure et al. 1998), supporting pharmacological data showing that 5-HT release in raphe neurons of the rat is controlled by 5-HT<sub>1A</sub> and 5-HT<sub>1B/D</sub> receptors (Davidson and Stamford 1995; Pineyro and Blier 1996) as well as electrophysiological findings that 5-HT<sub>1A</sub> and 5-HT<sub>1B</sub> receptors control the firing of dorsal raphe serotonergic neurons in mice (Evrd et al. 1999). The fact that 5-HT<sub>1B</sub> and 5-HT<sub>1D</sub> receptors are able to form homo- and heterodimers (Xie et al. 1999) may further enhance the complexity of the 5-HT<sub>1B/D</sub> pharmacology. In this context it is of interest to know whether 5-HT<sub>1B</sub> and 5-HT<sub>1D</sub> receptors coexist in the same raphe nuclei and in the same neurons in the human brain.

Materials and methods

In order to study the presence of h5-HT<sub>1BD</sub> Receptors in the human dorsal raphe, we used in situ hybridisation. Antisense oligonucleotide probes (AGGGTAGGGAGATGGACCTGCTGGTAAAATGTAAGCTCTGGCGG) corresponding to nucleotides 163–203 of the h5-HT<sub>1B</sub> receptor and (AGCGCTGGAGGTCTGGGATTCCTCCTGGCGG) corresponding to nucleotides 112–153 of the h5-HT<sub>1D</sub> sequence and the corresponding sense oligonucleotides (Göthert and Schlicker 1997).

Results

The dorsal raphe was identified as an accumulation of neurons ventral and ventrolateral to the aqueduct and fourth

Fig. 1 Silver-stained transverse section through the human mesencephalon at the level of the isthmus showing four of the five known subnuclei of the human dorsal raphe (DR; d dorsal, if inter-fascicular, v ventral, vl ventro-lateral, Aq aqueduct, CIF compact inter-fascicular nucleus, mlf medial longitudinal fascicle). The dark scattered neurons below the CIF and medial portion of the mlf may belong to the dorsal aspects of the median raphe. Bar=0.5 mm