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Short communication

des-AA-^{1,2,5}[D-Trp^{8}, IAmp^{9}]Somatostatin-14 allows the identification of native rat somatostatin sst$_1$ receptor subtype

Philippe Leroux *, Christine Bucharles, Evelina Bologna, Hubert Vaudry

Laboratory of Cellular and Molecular Neuroendocrinology, European Institute for Peptide Research (IFRMP No. 23), INSERM U413, UA CNRS, University of Rouen, 76821 Mont-Saint-Aignan, France

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Abstract

Somatostatin exerts multiple activities by interacting with at least five different receptor subtypes (sst$_{1-5}$). The affinity of des-AA^{1,2,5}-[D-Trp^{8}, IAmp^{9}]somatostatin-14 (CH-275) was studied by competition experiments using the non-selective radioligand [^{125}I][Leu^{8}, D-Trp^{22}, Tyr^{25}]somatostatin-28 in areas of the rat brain and pituitary known to express identified receptor subtypes. In the cerebellar nuclei and cerebral cortex, which possess the somatostatin sst$_1$ receptor subtype, CH-275 exhibited a moderate affinity (IC$_{50}$: 10–50 nM). Conversely, in the hippocampus, immature cerebellum and pituitary which contain different subsets of receptors mRNAs (sst$_{2-5}$), the IC$_{50}$ values were >1 μM. These data indicate that CH-275 is an appropriate ligand for the identification of native rat somatostatin sst$_1$ receptor subtype. © 1997 Elsevier Science B.V.

Keywords: Somatostatin binding site; CH-275; CH-288; Cerebellar nucleus

1. Introduction

The neuropeptide somatostatin exerts biological activities as a hormone, a neurohormone and a neurotransmitter in many organs via specific receptors (Epelbaum et al., 1994; Schusdziarra, 1996). The five cloned somatostatin receptors (sst$_{1-5}$) display specific binding and pharmacological characteristics when transfected in tumor cells (Hoyer et al., 1995). These recombinant receptors belong to one of the two classes of binding sites previously identified on the basis of their high and low affinity for the short-chained somatostatin analog octreotide, respectively. The first class, referred to as SRIF$_1$, appears to comprise sst$_2$, sst$_3$ and sst$_5$ receptor subtypes. The other class, referred to as SRIF$_2$, appears to comprise the sst$_1$ and sst$_4$ receptor subtypes (Hoyer et al., 1995). Hexapeptide and octapeptide somatostatin analogs have been identified as selective, high affinity ligands of sst$_2$, sst$_3$ and sst$_5$ receptors transfected in tumor cell lines (Raynor et al., 1993a,b; Patel and Srikant, 1994; Bruns et al., 1996). Recently, the undecapeptide des-AA^{1,2,5}-[D-Trp^{8}, IAmp^{9}]somatostatin-14 analog (CH-275) and its tyrosylated derivative ([Tyr^{3}]CH-275; CH-288) have been shown to bind selectively to human sst$_1$ receptor transfected in the Chinese hamster ovary (CHO-DG44) or in the Simian fibroblast-like (COS-7) tumor cell lines (Liapakis et al., 1996b). No specific ligand for the sst$_4$ receptor has been identified so far.

In a previous study, we have characterized sst$_1$ receptors in the rat cerebellar nuclei, by a binding approach (Bucharles et al., 1994). The present study was aimed at determining the selectivity of the newly described hsst$_1$ selective ligands CH-275 and CH-288 to rat native somatostatin receptors.

2. Material and methods

2.1. Chemicals

Somatostatin-14 was kindly provided by Dr. D. Djian (Sanofi, Paris, France). [Leu^{8}, D-Trp^{22}, Tyr^{25}]somatostatin-28 (LDTT-S28), CH-275 and CH-288 were generous gifts from Drs. C. Hoeger and J. Rivier (The Salk Institute, La Jolla, CA, USA).

* Corresponding author. Tel.: (33-2) 3514-6743; Fax: (33-2) 3514-6946; e-mail: philippe.leroux@univ-rouen.fr

2.2. Animals

Animals were kept under a 12:12 h light:dark cycle, with water and food ad libitum. Adult male rats (200–250 g), or 8 day-old (P8) pups were killed by decapitation and the tissues were collected, frozen (−30°C) and sectioned (12 μm) in a cryomicrotome. Brains from fetuses were collected at embryonic day 21.

2.3. Binding studies

Binding experiments and quantification of autoradiograms were performed as previously described (Leroux et al., 1991; Bucharles et al., 1994). Competition curves of [^{125}I]LDTT-S28 binding by somatostatin and analogs were performed on tissue sections. The binding was measured in autoradiograms in areas of the sections containing identified mRNAs or characterized somatostatin receptor subtypes: sst_1 in the cerebellar nuclei (Bucharles et al., 1994; Pérez et al., 1994); sst_2 in the external granule cell layer of the cerebellum from P8 pups (Viollet et al., 1997) and in the intermediate zone of the cerebral cortex of E21 fetuses (Leroux et al., 1995); sst_{1-5} in the anterior pituitary (Day et al., 1995; O'Carroll and Krempels, 1995), in the cortical plate of fetuses and in layers II–VI of the adult cerebral cortex (Leroux et al., 1995; Thoss et al., 1996) and sst_{1-4} in the hippocampus (Pérez et al., 1994; Harrington et al., 1995). Kinetic parameters of competition studies were determined using Sigmaplot software (Jandel Scientific, Corte Madera, CA, USA).

3. Results

The specific binding of [^{125}I]LDTT-S28 was higher than 80% in all structures studied except in the superficial layers II–III of the adult cortex (53%). Results of competition experiments of [^{125}I]LDTT-S28 binding by somatostatin, CH-275 and CH-288 are summarized in Table 1. Somatostatin exhibited the highest affinity (IC_{50}: 0.39 ± 0.17 to 0.45 ± 0.05 nM) in structures containing only sst_2 receptors (cerebellum external granule cell layer and intermediate zone of the cortex) and a lower affinity (IC_{50}: 2.30 ± 0.27 to 3.50 ± 0.32 nM) in structures containing sst_1 receptors (cerebellar nuclei). In areas containing mixtures of receptor subtypes (Ammon's horn of hippocampus, dentate gyrus, adult cerebral cortex, anterior pituitary) the IC_{50} values ranged from 0.81 ± 0.06 to 3.50 ± 0.32 nM and the Hill coefficients calculated from competition curves were inferior to 1 (not shown).

CH-275 and CH-288 were equipotent in most instances. In the hippocampus only, CH-275 at micromolar concentrations inhibited a larger proportion of the binding than CH-288. In tissues lacking or containing a low proportion

Table 1
IC_{50} values (nM) of somatostatin, CH-275 and CH-288 and percentage inhibition by 1 μM of CH-275 and 1 μM CH-288 on [^{125}I]LDTT-S28 binding in various brain areas and anterior pituitary in rat

	IC_{50} (nM)			Specific binding inhibited	
	Somatostatin	CH-275	CH-288	CH-275 (1 μM)	CH-288 (1 μM)
Cerebellar nuclei					
Lateral nucleus	2.30 ± 0.27 (6) [a]	23.5 ± 3.3 (14)	31.5 ± 5.0 (12)	94.2 ± 1.3	92.7 ± 0.7
Interposate nucleus	2.63 ± 0.20 (6)	35.5 ± 5.2 (14)	33.0 ± 6.1 (12)	91.5 ± 1.7	92.3 ± 1.5
Medial nucleus	3.12 ± 0.24 (6)	42.9 ± 7.2 (9)	44.7 ± 11.5 (12)	90.5 ± 1.7	89.3 ± 1.2
Fetal cerebral cortex					
Intermediate zone	0.45 ± 0.05 (6)	> 1000	> 1000	15.1 ± 7.1	8.9 ± 6.0
Cortical plate	0.73 ± 0.19 (6)	49.5 ± 11.2 (6)	46.6 ± 19.6 (6)	69.9 ± 4.5	72.3 ± 1.4
Neonatal cerebellum					
External granule cell layer	0.39 ± 0.17 (5)	> 1000	> 1000	15.5 ± 3.8 (6)	0
Cerebral cortex					
Layers II–III	0.81 ± 0.06 (6)	34.1 ± 6.2 (6)	13.7 ± 2.2 (6)	90.1 ± 3.4	87.1 ± 2.6
Layers V–VI	1.09 ± 0.12 (6)	43.7 ± 7.1 (6)	17.3 ± 5.3 (6)	76.8 ± 2.6	70.3 ± 2.5
CA1 field of hippocampus					
Stratum oriens	0.83 ± 0.31 (3)	> 1000	> 1000	45.7 ± 3.6	25.2 ± 5.6
Stratum radiatum	1.10 ± 0.24 (3)	> 1000	> 1000	51.9 ± 1.7	28.6 ± 3.9
Stratum pyramidale	0.88 ± 0.26 (3)	> 1000	> 1000	44.8 ± 11.5	14.2 ± 3.2
Dentate gyrus					
Stratum granulosum	3.50 ± 0.32 (3)	> 1000	> 1000	22.8 ± 4.5	17.0 ± 1.8
Anterior pituitary	1.48 ± 0.31 (4)	> 1000	> 1000	31.5 ± 6.5	24.5 ± 2.5

[a] Number of determinations.

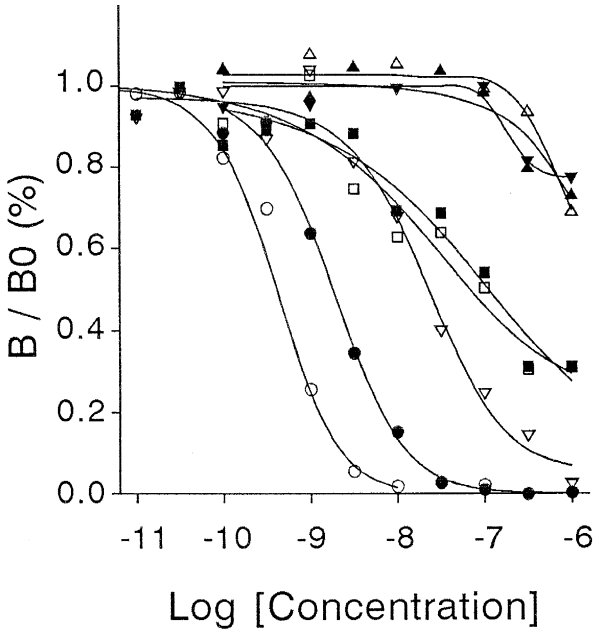

Fig. 1. Competition curves showing the displacement of [^{125}I]LDTT-S28 binding by somatostatin in the cortical intermediate zone (○) and the lateral cerebellar nucleus (●) and by CH-275 in the lateral cerebellar nucleus (▽), the cortical intermediate zone (▼), the cortical plate (□), the cortical layers V–VI (■), the stratum oriens of the hippocampal field CA1 (△) and the pituitary (▲).

of sst_1 receptors (cerebellum external granule cell layer and intermediate zone of the cortex, hippocampus and anterior pituitary), the potencies of CH-275 and CH-288 were low ($IC_{50} > 1$ μM). In these areas neither CH-275 nor CH-288 used at a concentration of 1 μM were able to displace more than 50% of [^{125}I]LDTT-S28 binding (Fig. 1). In structures containing sst_1 receptors (deep cerebellar nuclei, cortical plate, adult cerebral cortex) the IC_{50} values for CH-275 and CH-288 were comprised between 13.7 and 49.5 nM (Table 1). The inhibitory effects of CH-275 and CH-288 (1 μM) on the binding of [^{125}I]LDTT-S28 ranged from 69.9 to 76.8% in the cortical plate and cortical layers V–VI and from 89.3 to 94.2% in cerebellar nuclei (Table 1, Fig. 1).

4. Discussion

The present data show that CH-275 and CH-288 behave as selective ligands for native rat sst_1 receptor subtypes. Both ligands have low affinity for native sst_2 as shown by their inability to displace [^{125}I]LDTT-S28 binding in the P8 rat external granule cell layer of the cerebellum and the intermediate zone of E21 rat cortex, two regions of the developing brain which only contain sst_2 receptors (Leroux et al., 1995; Viollet et al., 1997). As no specific ligand for the rat sst_3 receptor is available yet, we could not select any brain structure to study the affinity of CH-275 and CH-288 for the native sst_3 receptor subtype. However, in a previous report we have shown that CH-275 has a low affinity (EC_{50}: 577 ± 140 nM) for recombinant rat sst_3 receptors expressed in human embryonic kidney (HEK-293) cells (Viollet et al., 1997). The sst_4 receptor is considered to be the major receptor subtype expressed in the hippocampus (Bito et al., 1994; Harrington et al., 1995) and sst_5 appears to be the major receptor type in the anterior pituitary since somatostatin-28 exhibits a higher affinity than somatostatin-14 for pituitary receptors (Srikant and Patel, 1981). In these tissues, CH-275 and CH-288 did not compete for [^{125}I]LDTT-S28 binding indicating that the two analogs have low affinity for rat sst_4 and sst_5 receptor subtypes, and that sst_1 receptors only represent a minor proportion of the binding sites. The selectivity of CH-275 and CH-288 for sst_1 versus sst_2, sst_4 and sst_5 receptors was at least 30 fold in as much as micromolar concentrations of each ligand could not displace more than 50% of the binding of [^{125}I]LDTT-S28 in areas containing these receptors (Table 1). The selectivity for sst_1 versus sst_3 receptor expressed in HEK-293 cells was about 10–15 fold.

The IC_{50} measured for both analogs were in the same order of magnitude as those measured in rat sst_1 receptors expressed in transfected HEK-293 cells (45.7 ± 12.0 nM) (Viollet et al., 1997). These values were 10-fold lower than those reported for the human and mouse recombinant sst_1 receptors expressed in CHO-DG44 or COS-7 cell lines, respectively (Liapakis et al., 1996a,b). Since the sequences of rat and human/mouse only differ by 5/6 amino-acids (out of 391 residues) located outside the domains involved for the high affinity binding of somatostatin (Fitzpatrick and Vandlen, 1994; Liapakis et al., 1996a), the different affinities of rat and human/mouse sst_1 receptors for CH-275 are more likely attributable to different experimental conditions than to variation in their primary structures.

In conclusion, CH-275 and CH-288 are selective ligands for the native sst_1 receptor subtype in rat. These ligands make it possible for the first time, to unambiguously identify native sst_1 receptors but their modest affinities prevent from using them as radioactive probes for the study of this receptor.

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