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Three apparent receptor subtypes for the endothelin/sarafotoxin family

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Competition binding experiments performed with ^{125}I-sarafotoxin (SRTX)-b and SRTX-b, SRTX-c and endothelin (ET-1 and ET-3) using homogenates of rat right and left atria, aorta, uterus, cerebellum and caudate putamen indicated heterogeneity of the ET/SRTX receptor. The evidence pointed to the existence of three receptor subtypes: a high-affinity ET-1/SRTX-b subtype typical of smooth muscle (E-S_α receptor), a high-affinity SRTX-c subtype typical of the cerebellum (E-S_β receptor), and a less selective subtype typical of the caudate putamen that binds all of these peptides with high affinity (E-S_γ receptor).

Sarafotoxin; Endothelin; Receptor subtype; (Rat, Brain, Aorta, Heart, Uterus)

1. INTRODUCTION

We recently characterized a high-affinity receptor for the cardiotoxic vasoconstrictor peptides, the sarafotoxins (SRTX), and for the vasoconstrictor peptides, endothelins (ET), in the rat heart and brain, and demonstrated the association of this receptor with phosphoinositide hydrolysis [1–5]. The sarafotoxins (SRTX-a, SRTX-b and SRTX-c [6,7]) and their structurally related mammalian vasoconstrictor peptides the endothelins (ET-1, ET-2, ET-3) [8,9] (see fig.1) were found to share many similarities in binding to their receptor, in their ability to induce phosphoinositide hydrolysis, regional distribution ([10] and references therein) and vasoconstrictive activities [8,9,11]. In spite of their marked similarities in structure (fig.1) and activities, especially in the case of ET-1 and SRTX-b [11,12], some differences were noted between the activities and binding properties of these two peptides and those of the other peptides of the ET/SRTX family [2,3,10]. Most important were the pronounced vasodilatory activities of ET-3 [13] and of SRTX-c [11] as well as the significantly higher binding affinities of SRTX-b and of ET-1 for their receptors in the cerebral cortex and in the caudate putamen than in the cerebellum or in the atria [2,3,10]. If such variations are due to regional heterogeneity of the receptors, it should be possible to detect significant differences among different tissues with respect to binding profiles of peptides of the ET/SRTX family. The results reported here strongly suggest the existence of ET/SRTX receptor subtypes.

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Abbreviations: SRTX, sarafotoxin; ET, endothelin; E-S_α, a high-affinity ET-1/SRTX-b subtype; E-S_β, a high-affinity SRTX-c subtype; E-S_γ, subtype that binds all of the peptides under investigation with high affinity

2. EXPERIMENTAL

SRTX-a, SRTX-b, and SRTX-c were purified to homogeneity from the venom of the snake *Atractaspis enggadensis* [6,7]. ^{125}I-SRTX-b (2.5×10^{17} cpm/mol) was prepared as described [2]. ET-1 was purchased from American Peptide Company Inc.

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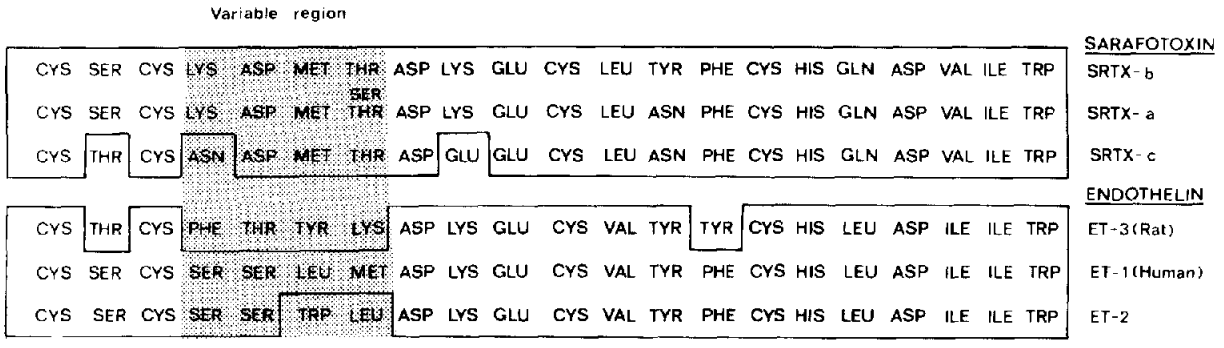


Fig.1. Structure of naturally occurring sarafotoxins [6,7] and endothelins [8,9].

(Santa Monica, CA) and ET-3 from Peninsula Labs Europe Ltd (Merseyside, England). Tissue preparation and assays for ^{125}I-SRTX-b binding were as described [1–3].

Adult male Charles River derived (CD) rats were decapitated, the required tissues removed, dissected and homogenized in 25 mM Tris-HCl buffer, pH 7.4 (0.05 g tissue/ml buffer) containing protein inhibitors (5 U/ml aprotinin, 5 μg/ml pepstatin A, 0.1 mM phenylmethylsulfonyl fluoride, 3 mM EDTA and 1 mM EGTA). Aliquots (50 μl) of tissue homogenates (containing 150 μg protein) were incubated at 25°C for 60 min with various concentrations of ^{125}I-SRTX-b (direct binding) or with 2.5 nM ^{125}I-SRTX-b and various concentrations of peptides (competition experiments) in 25 mM Tris-HCl buffer, pH 7.4 (total volume 200 μl). Reactions were terminated by the addition of 3 ml of ice-cold Tris-HCl buffer and filtration under vacuum through GF/C filters; adsorption of ^{125}I-SRTX-b to the filters under these assay conditions was negligible. The filters were then washed twice with 3 ml of buffer and their radioactivity was estimated in scintillation counter (LKB, 1209 Rackbeta). Assays were performed in triplicate (total binding) together with triplicate samples containing 1 μM SRTX-a (nonspecific binding).

3. RESULTS

In line with our previous experiments [1–4] we observed saturable high-affinity binding of ^{125}I-SRTX-b in the rat cerebellum (K_d = 4 nM), caudate putamen (K_d = 0.3 nM) and heart (K_d = 4.0 nM). Interestingly, the various regions of the heart showed significant differences in binding capacity: it was higher in the right atria than in the left (225 ± 35 vs 150 ± 25 fmol/mg protein) and lowest in the ventricles (18.5 ± 4.5 fmol/mg protein). There were no such regional differences in the K_d values for ^{125}I-SRTX-b (6, 4.9 and 4.7 nM for the right and left atria and the ventricles, respectively). Thus, as judged by the direct binding of ^{125}I-SRTX-b, the receptors in the caudate putamen and the cortex [2] appeared to be distinct from those in the cerebellum and the atrium.

A more sensitive way to examine the possible existence of receptor heterogeneity is by employing a variety of ET/SRTX analogs in competition binding experiments to different tissues. We accordingly carried out such experiments in muscle tissues (right and left atria, aorta and uterus), and in two brain regions (cerebellum and caudate putamen). Results of a typical experiment demonstrate that the competition patterns of the various peptides differ markedly among the right atrium, the aorta, the cerebellum, and the caudate putamen (fig.2). In the right atria SRTX-b and ET-1 are the most potent inhibitors of the binding of ^{125}I-SRTX-b to its receptor, ET-3 is a moderate inhibitor and SRTX-c is at least 20 times less potent than either SRTX-b or ET-1. The IC_{50} values (IC_{50} = concentration of peptide resulting in 50% inhibition of the binding of 2.5 nM ^{125}I-SRTX-b) for the right atria were similar to those for the left atria and the uterus (table 1). In the aorta, as in the atria, ET-1 and SRTX-b were found to be potent inhibitors of ^{125}I-SRTX-b binding to its receptor; however, ET-3 did not inhibit the binding even at a concentration as high as 2 μM, and the IC_{50} of SRTX-c was also high (1.1 μM). Thus the two latter peptides, while capable of inhibiting ^{125}I-SRTX-b binding to its receptor in the atria (IC_{50} 60–70 nM to 100–200 nM for ET-3 and SRTX-c, respectively), are almost ineffective in competing with ^{125}I-SRTX-b for binding to its receptor in the aorta. The two brain regions examined here exhibited profiles which differed from each other and from those observed in the atria or the aorta (fig.2, table 1). In the caudate putamen all the

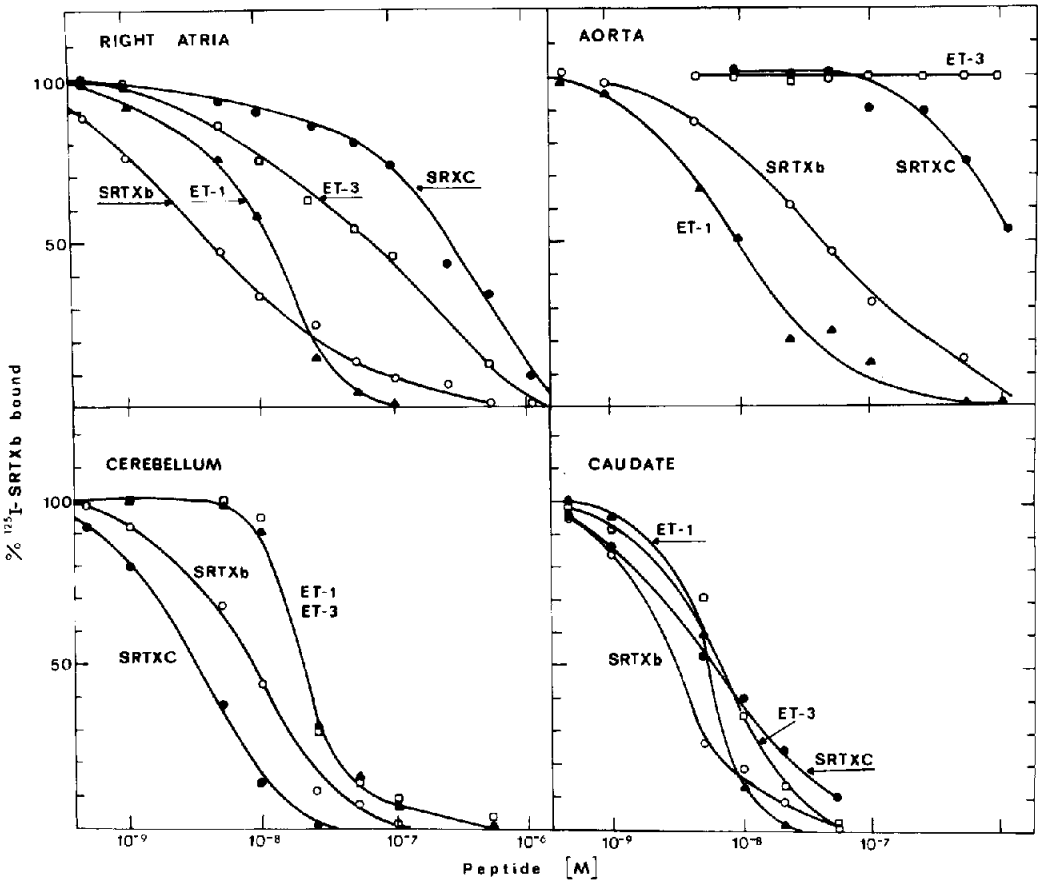


Fig.2. Concentration dependent inhibition of ^{125}I-SRTX-b binding to its receptor in various tissues by ET/SRTX peptides. Binding assays were as described in section 2 using 2.5 nM ^{125}I-SRTX-b and various concentrations of the peptides.

Table 1

IC_{50} values of ET/SRTX peptides determined by inhibition of ^{125}I-SRTX-b binding to its receptors in various tissues

	IC_{50} (nM)					
	Right atria	Left atria	Aorta	Uterus	Cere-bellum	Caudate putamen
ET-1	11	13	10	16	42	5
SRTX-b	7.5	7	40	6	8	2.5
ET-3	68	75	>2000	230	60	10
SRTX-c	270	110	1100	700	3.5	6

IC_{50} values (IC_{50} = concentration of peptide resulting in 50% inhibition of the binding of 2.5 nM ^{125}I-SRTX-b) represent the mean values of two to three separate experiments similar to those shown in fig.2

tested peptides showed comparable potencies in inhibiting the binding of ^{125}I-SRTX-b to its receptor (IC_{50} = 2.5–10 nM), whereas in the cerebellum SRTX-c was the most potent inhibitor of binding (IC_{50} = 3.5 nM) (table 1).

4. DISCUSSION

The possible existence of receptor subtypes for the ET and SRTX peptide families has already been discussed recently by us [2,3,10]. In this study we employed ^{125}I-SRTX-b as a probe for the detection of ET/SRTX receptor heterogeneity in various murine tissues. Using this ligand and five

different peptides of the ET/SRTX family we found remarkable differences between the peptides in their binding potencies in the different tissues. Receptors of smooth muscle, which we term E-S_α receptors, displayed a high affinity for ET-1 and SRTX-b and a low affinity for ET-3 and SRTX-c. A significantly higher affinity for SRTX-c and a lower but comparable affinity for ET-1, SRTX-b and ET-3 were shown by the cerebellar receptors, which we designate as E-S_β receptors. A third pattern of binding was observed in the caudate putamen where all the peptides tested (ET-1, SRTX-b, ET-3 and SRTX-c) bind to the receptor with comparable high affinities. These receptors, which are less selective than either of the other two, we termed E-S_γ receptors. It seems likely that the variable region of the ET/SRTX peptides [10] (fig.1) plays a major part in determining their propensity to interact with a particular receptor subtype. It should be noted that the approach employed in this study enabled us to distinguish between apparent receptor subtypes by virtue of the observed differences in their structure-binding relationships in the various tissues. Future studies should be aimed at determining whether the apparent receptor subtypes described here are associated with different second messenger systems. It has already been shown that one of them, which binds ET-1 and SRTX-b with high affinity [1,2,14,15] and appears to correspond to the E-S_α subtype, is associated with phosphoinositide hydrolysis [1,3,4] and Ca^{2+} mobilization [16,17]. It would also be of interest to identify the endogenous agonist for the E-S_β receptors, for which SRTX-c appears to be a selective agonist, and to determine whether or not there exists a selective natural agonist for E-S_γ receptor. Also worth investigating is the relative abundancy of each of the apparent ET/SRTX receptor subtypes in various brain and peripheral tissues.

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