Volume 242, number 2, 387-390 FEB 06672 January 1989

Sarafotoxin receptors mediate phosphoinositide hydrolysis in various rat brain regions

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Received 31 October 1988; revised version received 13 November 1988

Sarafotoxin-b, a potent snake vasoconstrictor peptide homologous to the mammalian endothelial vasoconstrictor endothelin, induces phosphoinositide (PI) hydrolysis in various brain regions of the rat. Sarafotoxin-b induced PI hydrolysis is largely independent of extracellular Ca^{2+} and is detected in all brain regions where toxin-binding sites are found. These results point to the existence of a hitherto undetected neuroreceptor associated with the PI cycle.

Snake venom; Sarafotoxin; Receptor; Endothelin; Phosphoinositide; (Rat brain)

1. INTRODUCTION

SRTXs (in our previous paper we used SRT (without the X) as acronym for the sarafotoxin), a group of 21-residue cysteine-rich peptides [1,2] isolated from the venom of the snake *Atractaspis engaddensis*, were shown to activate the hydrolysis of phosphoinositides in a dose-dependent manner [3]. Thus, sarafotoxins appear to interact with and activate a specific phosphoinositide (PI) phosphodiesterase system, thereby triggering the PI cycle. This cycle is a major second-messenger system in brain and peripheral tissues; its triggering by hormones and neurotransmitters results in the hydrolysis of phosphatidyl inositol bisphosphate to IP_3 and diacylglycerol [4]. IP_3 activates the release of calcium from intracellular stores [4], and diacylglycerol activates protein kinase C [5]. We therefore suggested that SRTXs might induce an increase in intracellular Ca^{2+} levels, which could account for their vasoconstrictor activity and for the disturbances caused in the A-V conducting system [3,6].

As neither the binding nor the PI hydrolysis induced by SRTXs is affected by blockers or activators of known receptors or ion channels [3], it seems that this group of peptides might interact with a specific PI-receptor system which operates through a putative 'SRTX-like' neuromodulator, both in rat heart and rat brain. Support for this suggestion comes from the recent discovery of endothelin [7], an endogenous 21-residue cysteine rich vasoconstrictor peptide present in mammalian endothelial cells and showing a high degree of homology to SRTXs [3].

In the present communication we report on the localities of SRTX-b-induced phosphoinositide hydrolysis and their relationships with the densities of ^{125}I-SRTX binding sites.

2. EXPERIMENTAL

SRTX-b was isolated and purified to homogeneity from the venom of the snake *A. engadensis* as described [1,2].

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Abbreviations: SRTX, sarafotoxin; PI, phosphoinositide; IP_1, inositol-1-phosphate; IP_2, inositol-1,4-bisphosphate; IP_3, inositol-1,4,5-trisphosphate; G_o, guanine nucleotide binding protein present in the brain; G_p, guanine nucleotide binding protein associated with phospholipase C

Published by Elsevier Science Publishers B.V. (Biomedical Division)
00145793/89/$3.50

Preparation of brain slices has been described in detail [8]. Briefly, adult male Charles River derived (CD) rats were decapitated, their brains removed, dissected and sliced with a Sorval TC-2 tissue sectioner (200 × 200 μm). The formation of $[^3H]IP_3$, $[^3H]IP_2$ and $[^3H]IP_1$ in the tissue slices was assayed [9] by the method of Berridge [10]. Slices were prelabeled (60 min) with 60 μCi/ml of [^{3}H]inositol (18.7 Ci/mmol, Amersham) in Krebs medium, washed 3 times with 5 mM inositol in the same medium. Packed slices (50 μl) were then incubated in Krebs medium (total volume 250 μl) containing 10 mM LiCl with and without SRTX-b. The reaction was terminated after 30 min (or as indicated in the text) by the addition of 1 ml chloroform/methanol (1:2), followed by 0.35 ml chloroform and 0.35 ml H_2O. The water-soluble products were separated chromatographically on Dowex columns [10] and counted with corrections for quenching. A sample from the lipid extract was also counted. All assays were performed in triplicate. Data are expressed in terms of [^{3}H]inositol phosphate formed as a percentage of total labeled [^{3}H]inositol lipids. Zero time blanks were subtracted.

Protein was determined by the Lowry method using bovine serum albumin as a standard.

3. RESULTS

Since the binding of SRTXs in the rat atrium was coupled to the hydrolysis of phosphoinositides [3], we examined SRTX-b-induced PI hydrolysis in various regions of the rat brain. Initial experiments explored the time course of the formation of [^{3}H]inositol phosphates in the presence of 10 mM LiCl in [^{3}H]inositol-labeled tissue slices from the cerebellum and cortex. In both tissues, 0.1 μM SRTX-b induced a time-dependent increase in the formation of $[^3H]IP_1$: IP_1 was detected after a short lag period (5–7 min) and continued to increase almost linearly for at least 30 min (fig.1). The levels of $[^3H]IP_2$ were very low compared to those of IP_1, while $[^3H]IP_3$ was barely detectable (levels recorded were within the limits of experimental error).

SRTX-induced PI hydrolysis in the cerebellar and cerebral cortical tissue slices was not blocked by ligands of known receptors or ion channels (fig.1). Other experiments showed that PI hydrolysis could be induced by SRTX-b in a calcium-free medium, with and without 1 mM EGTA (fig.2). Even though the efficacy of the toxin was reduced under these conditions, the ratio of the induced to basal hydrolysis remained unchanged (fig.2). We could not, however, demonstrate SRTX-b-induced PI hydrolysis in homo-

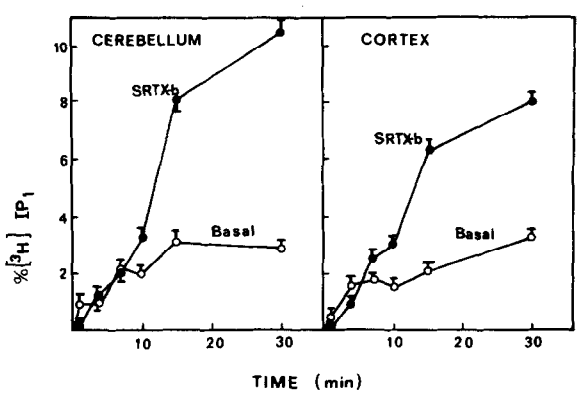

Fig.1. Time courses and specificity of SRTX-b-induced PI hydrolysis in cerebral and cerebellar tissue slices. Tissue slices from both brain regions were prelabeled with [^{3}H]inositol and subjected to the PI hydrolysis assay in the absence and in the presence of 10^{-7} M SRTX-b, as detailed in section 2. The data represent basal and toxin-induced accumulation of $[^3H]IP_1$, expressed in terms of $[^3H]IP_1$ formed as a percentage of total [^{3}H]inositol lipids. Zero time blanks were subtracted. Values are means ± SD (vertical bars) of triplicate samples. The following substances or ions failed to inhibit induction of PI hydrolysis by SRTX-b (10^{-7} M): atropine (10 μM), curare (10 μM), haloperidol (10 μM), naloxone (10 μM), propranolol (10 μM), yohimbine (10 μM), prazosin (0.1 μM), allersan (10 μM), tetrodotoxin (1 μM), tetraethylammonium (1 mM), nimodipine (1 μM), verapamil (1 μM), cobalt (1 mM), manganese (1 mM).

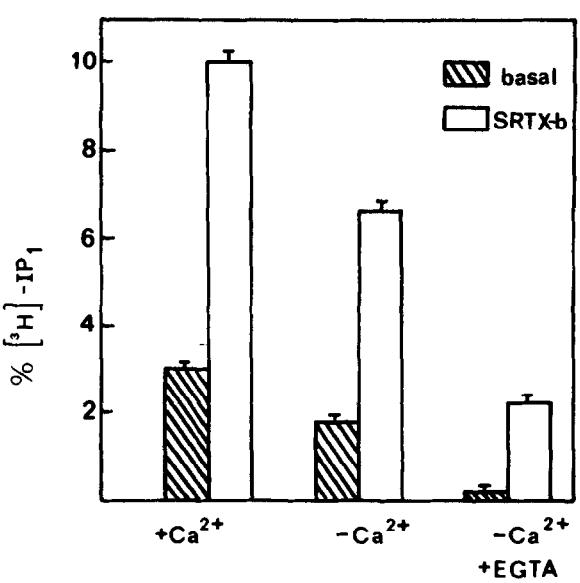

Fig.2. Induction of PI hydrolysis by SRTX-b in the absence and in the presence of Ca^{2+}. PI hydrolysis was determined in cerebellar tissue slices (30 min incubation time) as detailed in the legend to fig.1. The experiments were conducted with or without 1 mM $CaCl_2$, in the presence or absence of 1 mM EGTA. Toxin concentration was 0.1 μM.

Table 1

Regional distribution of ^{125}I-SRTX-b binding sites and of SRTX-b-induced PI hydrolysis[a]

Brain tissue	^{125}I-SRTX-b binding sites (fmol/mg protein)	SRTX-b-induced PI hydrolysis, toxin/basal
Cerebellum	850 ± 50	3.5 ± 1
Hypothalamus	440 ± 10	7.4 ± 2
Thalamus	450 ± 50	3.8 ± 1
Brain stem	320 ± 10	4.2 ± 1
Olfactory bulb	200 ± 50	4.2 ± 0.2
Spinal cord	140 ± 20	2.5 ± 0.2
Hippocampus	150 ± 10	5.2 ± 1
Caudate putamen	100 ± 20	7.7 ± 1
Cerebral cortex	72 ± 10	2.5 ± 1

[a] Data for binding densities were taken from [11]. Values are means ± SD of five separate determinations. PI hydrolysis is expressed as the ratio of SRTX-b (10^{-7} M) induced formation of [^{3}H]IP$_1$ (30 min) to basal (means ± SD, three separate determinations) determined under the assay conditions specified in section 2

genates of these tissues. The homogenates were derived from tissue slices prelabeled with [^{3}H]inositol, and homogenized in a Ca^{2+}-free medium containing 1 mM EGTA. In the resulting labeled membranes we were able to demonstrate a time-dependent increase in IP_1 in the presence of 1 mM Ca^{2+} (but not in its absence) and NaF, but not SRTX-b-stimulated PI hydrolysis. Thus, under the conditions employed here, SRTX-b-induced PI hydrolysis in the brain was detectable only in tissue slices.

We then proceeded to measure the SRTX-b-induced formation of IP_1 in tissue slices from various brain regions. The results (expressed as toxin-induced PI hydrolysis over basal) are summarized in table 1 along with the density of ^{125}I-SRTX binding sites.

4. DISCUSSION

In this study we characterized SRTX-b-induced PI hydrolysis in rat brain and its regional localities. In all brain regions studied we detected both SRTX-induced PI hydrolysis and ^{125}I-SRTX binding [3,11]. Our results suggest that the SRTX-b-binding sites are associated at least in part with the important PI/acylglycerol second-messenger system [4,5] of the brain. Both in the brain and in the peripheral tissues, this system can be triggered by external stimuli produced by neurotransmitters, hormones, growth factors and chemoattractants which interact with their respective receptors. It may also be stimulated by the activation of ion channels leading to the entry of Ca^{2+} ions into cells [4]. Our observations on SRTX-b-induced PI hydrolysis in rat brain as well as in rat atrium [3] suggest that primary activity of SRTX-b is mediated via its own receptor and not via known ion channels since channel blockers such as tetrodotoxin, tetramethyl ammonium, nitrendipine, cobalt and manganese failed to inhibit its activity. Consistent with this notion is the finding that SRTX-b can induce PI hydrolysis in the brain even in the absence of Ca^{2+} and in the presence of EGTA. Thus, SRTX-b appears to activate a neuroreceptor which is coupled to the PI cycle. It could have been assumed to interact with a known component(s) of this cycle such as PI phosphodiesterase or G_p [12]. However, this assumption seems unlikely, since, in contrast to the SRTX-b binding sites, G_p and G_o are ubiquitous proteins (as reviewed in ref.13) and the latter is highly abundant in the brain. Moreover, the failure of SRTX-b (but not of Ca^{2+} or NaF) to induce PI hydrolysis in brain membranes prelabeled with [^{3}H]inositol (whereas Ca^{2+} or NaF did activate such hydrolysis in these membranes) argues against direct activation of PI phosphodiesterase by SRTX-b.

A comparison between the regional distribution of ^{125}I-SRTX receptors and the localities of SRTX-b-induced PI hydrolysis (table 1) shows no simple correlation between the two. For example, while the efficacy of the toxin is higher in the caudate than in the cerebellum, the density of ^{125}I-SRTX-b sites in the latter is by far much higher than in the former. This phenomenon could be explained by the existence of two or more subtypes of receptors. Such a suggestion is in line with two independent observations: (a) SRTX-b binds with a much higher affinity (K_d = 0.3 nM) to the receptors of the caudate and cerebral cortex than to the receptors of the cerebellum or the rat atrium (K_d = 3.5–4 nM). (b) In cultured epithelial cells, the closely related vasoconstrictor peptide endothelin was reported to induce Ca^{2+} influx but not PI hydrolysis [14]. Moreover, in other receptors coupled to PI hydrolysis such as the muscarinic

receptors, lack of correlation between receptor distribution and the efficacy of agonist-mediated PI hydrolysis is attributed to the existence of receptor subtypes.

As shown in fig.2, the extent of PI hydrolysis in the absence of Ca^{2+} (with EGTA) is lower than that observed in the presence of Ca^{2+}. The partial inhibition of IP_1 formation might be explained by assuming coupling between the toxin-receptor and a voltage-independent Ca^{2+} channel. The rise in Ca^{2+} resulting from Ca^{2+} influx could thus lead at least in part to PI breakdown and IP_1 formation. Alternatively, the Ca^{2+} dependent increase in PI hydrolysis could be related to secondary processes resulting from the main pathway of PI hydrolysis [4,5] induced by the toxin. In this context it should be noted that the results of Hirata et al. [14], who showed that endothelin caused both sustained and transient increases in intracellular Ca^{2+} levels (only the former increase is blocked by EGTA), are compatible with the above suggestion.

The present results and the high degree of homology between endothelin and SRTXs [3] may suggest that an endogenous substance 'SRTX-like' and/or 'endothelin-like' is present in the brain. Certain regions of the brain, such as the cerebellum, might contain high levels of this endogenous neuropeptide that interacts with the SRTX-receptor.

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