PROPERTIES OF TRICHOSPORIN-B-VIa-INDUCED CATECHOLAMINE SECRETION FROM BOVINE ADRENAL CHROMAFFIN CELLS

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Trichosporin (TS)-B-VIa, a peptide consisting of 19 amino acid residues and an amino alcohol, causes Ca^{2+} -dependent secretion of catecholamines from bovine adrenal chromaffin cells. The TS-B-VIa-induced secretion was greater under alkaline conditions and at a temperature of 37°C compared with that at 21° or 30°C. It was not observed when the peptide was eliminated from the incubation medium. These results strongly suggest that the stimulatory effect of TS-B-VIa on the secretion is reversible and dependent on the temperature and pH of the incubation medium.

KEY WORDS trichosporin; peptaibol; ion-channel; catecholamine secretion; chromaffin cell; *Trichoderma polysporum*

Trichosporin (TS)-B-VIa (Ac-Aib-Ala-Aib-Ala-Aib-Aib-Gln-Aib-Ile-Aib-Gly-Leu-Aib-Pro-Val-Aib-Aib-Gln-Gln-Pheol: Aib = α -aminoisobutyric acid, Pheol = phenylalaninol, Iva = isovaline)¹⁾ is one of the peptaibols²⁾ isolated from the culture broth of *Trichoderma polysporum* (Link ex Pers.) Rifai (strain TMI 60146). TS-B-VIa forms voltage-gated ion channels in lipid bilayers,³⁾ as do other peptaibols⁴⁾ and TS-Bs.⁵⁾ In addition, TS-B-VIa (2-5 μM) not only induces Ca^{2+} influx into excitable bovine adrenal chromaffin cells and results in Ca^{2+} -dependent catecholamine secretion in a concentration-dependent manner, but also causes Ca^{2+} influx into non-excitable C₆ glioma cells and bovine platelets, suggesting that TS-B-VIa *per se* also forms (Ca^{2+} -permeable) ion channels in biomembranes⁶⁾ as well as in artificial membranes. An influx of external Ca^{2+} into the chromaffin cells is essential for triggering the secretion of catecholamines from the cells.⁷⁾ The secretion, therefore, reflects the Ca^{2+} influx. In this study, we investigated the properties of TS-B-VIa-induced catecholamine secretion from the chromaffin cells.

We incubated the cultured bovine adrenal chromaffin cells for 7 min at 37°C without or with 3 μM TS-B-VIa (first incubation), washed them with Krebs-Ringer-Hepes (KRH) buffer (pH 7.4), and incubated again for an additional 7 min with or without 3 μM TS-B-VIa (second incubation). As shown in Table 1 (E), TS-B-VIa increased the secretion of catecholamines during the 14 min of incubation. After the first incubation with (Table 1, D) or without (Table 1, B) 3 μM TS-B-VIa for 7 min, the cells, which had been washed with the KRH buffer and incubated with 3 μM TS-B-VIa, showed the increased secretion during the second 7-min incubation (Table 1, D and B). In contrast, the cells, which had been washed and incubated with the buffer, did not secrete catecholamines during the second incubation (Table 1, C), indicating that the stimulatory effect of TS-B-VIa is reversible.

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Table 1
Reversibility of TS-B-VIa-induced Catecholamine Secretion from Bovine Adrenal Chromaffin Cells

		First incubation	Wash	Second incubation	
Addition		Catecholamine secretion (% of total)		Addition	Catecholamine secretion (% of total)
A	-	0.2 ± 0.1	+	-	0.2 ± 0.2
B	-	0.2 ± 0.1	+	TS-B-VIa	16.9 ± 2.1
C	TS-B-VIa	17.0 ± 2.4	+	-	0.2 ± 0.1
D	TS-B-VIa	18.9 ± 1.5	+	TS-B-VIa	12.0 ± 0.6
E	TS-B-VIa	-	-	-	26.6 ± 1.1

The cultured chromaffin cells in the dishes were incubated with (C and D) or without (A and B) $3 \mu\text{M}$ TS-B-VIa in Krebs-Ringer-Hepes (KRH) buffer (pH 7.4) for 7 min at 37°C (first incubation). The incubation medium was collected (A, B, C and D) and the cells in the dishes were washed with KRH buffer. The cells were then incubated with (B and D) or without (A and C) $3 \mu\text{M}$ TS-B-VIa in KRH buffer for an additional 7 min (second incubation). The cells were incubated with $3 \mu\text{M}$ TS-B-VIa for 14 min (E). Catecholamines in each collected reaction medium were determined according to the ethylenediamine condensation method. The amount of catecholamines was expressed as a percentage of the total cellular catecholamines ($30.1 \pm 0.9 \mu\text{g}$). Each value represents the mean $\pm$ S.D. of at least four experiments.

Fig. 1. Effect of Incubation Medium pH on TS-B-VIa-induced Catecholamine Secretion from the Chromaffin Cells

The cells were incubated with or without $3 \mu\text{M}$ TS-B-VIa in media of various pH for 10 min at 37°C . Catecholamines secreted from the cells were determined according to the ethylenediamine condensation method. The amount of catecholamines was expressed as a percentage of the total cellular catecholamines. Data are means $\pm$ S. D. from four experiments.

Figure 1 shows the effect of the pH of the incubation medium on the TS-B-VIa ($3 \mu\text{M}$)-induced secretion. At pH 6.0, the TS-B-VIa-induced secretion was 10% of total cellular catecholamines, at pH 7.4, 21%, and maximal (28%) at pH 8.0 (Fig. 1). Thus, the TS-B-VIa-induced catecholamine secretion increased with increasing pH of the incubation medium.

We examined the effect of incubation temperature on the TS-B-VIa-induced catecholamine secretion and Ca^{2+} influx. At 4°C , TS-B-VIa ($5 \mu\text{M}$) produced neither secretion (Fig. 2A) nor Ca^{2+} influx (Fig. 2B), while it increased both secretion (Fig. 2A) and Ca^{2+} influx (Fig. 2B) with an increase in the temperature (21 – 37°C). This indicates that the reduction of the TS-B-VIa-induced secretion at the lower temperatures is attributable to the inhibition of the Ca^{2+} influx rather than to the suppression of the secretory machinery in the cells at the lower temperatures, at least in this case.

Based on measurement of single-channel activity of TS-B-VIa in planar lipid bilayers, TS-B-VIa has been estimated to form an ion channel which is a bundle of four to nine peptide monomers.³⁾

Therefore, it is considered that the monomers of TS-B-VIa accumulate and insert into biomembranes to form pores permeable to ions. This study strongly suggests that the ion channel formation of

Fig. 2. Effects of Temperature on TS-B-VIa-induced Catecholamine Secretion from the Chromaffin Cells (A) and Ca²⁺ Influx into the Cells (B)
(A) The cells were incubated with or without 5 μM TS-B-VIa for 10 min at various temperatures. Catecholamines secreted from the cells were determined according to the ethylenediamine condensation method. The amount of catecholamines was expressed as a percentage of the total cellular catecholamines. (B) The cells were incubated with or without 5 μM TS-B-VIa in KRH buffer containing 1 μCi ⁴⁵Ca²⁺ for 10 min at various temperatures. Radioactivity in the cells was determined by liquid scintillation counting. The Ca²⁺ influx was expressed as nanomoles of Ca²⁺ per 2 × 10⁶ cells. Data are means ± S. D. from four experiments.

TS-B-VIa in biomembranes is reversible, and is affected by pH and temperature, which probably produces conformational changes in the peptide and influences the fluidity of the cell membranes.

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