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

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Trichovipin (TS) -B-VIa, a peptide consisting of 19 amino acid residues and an amino alcohol, causes Ca2+-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°C 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 trichophin; peptaibol; ion-channel; catecholamine secretion; chromaffin cell; Trichoderma polsporum

Trichovipin (TS)-B-VIa (Ac-Aib-Ala-Ala-Aib-Ala-Aib-Gln-Ala-Ile-Aib-Gly-Leu-Aib-Pro-Val-Aib-Aib-Gln-Gln-Pheol: Aib = α-aminoisobutyric acid, Pheol = phenylalaninol, Iva = isovaline) is one of the peptaibols isolated from the culture broth of Trichoderma polsporum (Link ex Pers.) Rifai (strain TMI 60146). TS-B-VIa forms voltage-gated ion channels in lipid bilayers, as do other peptaibols and TS-Bs. In addition, TS-B-VIa (2-5 μM) not only induces Ca2+ influx into excitable bovine adrenal chromaffin cells and results in Ca2+-dependent catecholamine secretion in a concentration-dependent manner, but also causes Ca2+ influx into nonexcitable C6 glioma cells and bovine platelets, suggesting that TS-B-VIa per se also forms (Ca2+-permeable) ion channels in biomembranes as well as in artificial membranes. An influx of external Ca2+ into the chromaffin cells is essential for triggering the secretion of catecholamines from the cells. The secretion, therefore, reflects the Ca2+ 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

<table>
<thead>
<tr>
<th></th>
<th>First incubation</th>
<th>Wash</th>
<th>Second incubation</th>
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<tbody>
<tr>
<td></td>
<td>Catecholamine secretion (% of total)</td>
<td></td>
<td>Catecholamine secretion (% of total)</td>
</tr>
<tr>
<td>A</td>
<td>-</td>
<td>0.2 ± 0.1</td>
<td>-</td>
</tr>
<tr>
<td>B</td>
<td>-</td>
<td>0.2 ± 0.1</td>
<td>+</td>
</tr>
<tr>
<td>C</td>
<td>TS-B-VIa</td>
<td>17.0 ± 2.4</td>
<td>+</td>
</tr>
<tr>
<td>D</td>
<td>TS-B-VIa</td>
<td>18.9 ± 1.5</td>
<td>+</td>
</tr>
<tr>
<td>E</td>
<td>TS-B-VIa</td>
<td>-</td>
<td>-</td>
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</tbody>
</table>

The cultured chromaffin cells in the dishes were incubated with (C and D) or without (A and B) 3 μM 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 μM TS-B-VIa in KRH buffer for an additional 7 min (second incubation). The cells were incubated with 3 μM 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 ± 0.9 μg). Each value represents the mean ± 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](image)

The cells were incubated with or without 3 μM 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 ± S. D. from four experiments.

Figure 1 shows the effect of the pH of the incubation medium on the TS-B-VIa (3 μM)-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 Ca²⁺ influx. At 4°C, TS-B-VIa (5 μM) produced neither secretion (Fig. 2A) nor Ca²⁺ influx (Fig. 2B), while it increased both secretion (Fig. 2A) and Ca²⁺ 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 Ca²⁺ 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.
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 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.

REFERENCES

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