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# Stimulation and Attenuation Effects of Neuroactive Amines on cAMP Production in Two-spotted Mites Membranes

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## Abstract

The interaction of biogenic amines including octopamine (OA), dopamine (DA), tyramine (TA), serotonin (5-HT), noradrenaline (NA) with octopamine / tyramine receptors and the resulting stimulation or attenuation effects of cyclic AMP (cAMP) production in vitro were investigated in the membrane homogenates of two-spotted spider mite, *Tetranychus cinnabarinus*. cAMP levels were determined by standard protein binding method. It's found that 100μM OA could cause around 310~420% increasing of cAMP comparing with the basal treatment, which was done with 10μM theophyline. The stimulation effect of OA could be inhibited completely by OA antagonist, like 100μM miaserin. The adenylate cyclase (AC) system could be activated by 10μM forskolin with an average stimulation of around 1200% enhancement of cAMP production. 5-HT and NA displayed no significant potential stimulating or attenuation effects on the AC activity. It's very interesting to find that DA, which has similar structure with octopamine except its m-hydroxyl group in the phenyl ring, could significantly inhibit the cAMP production at above 10μM concentration. Further study showed that $IC_{50}$ of DA as an antagonist behaviour was 25.1μM. It's apparent that 1mM DA could attenuate cAMP stimulation to a level below what the basal did. The attenuation of DA could be partially recovered from TA or DA antagonist yohimbine. More interestingly, with 10μM TA alone, it could cause about 40% increasing of cAMP. While 100μM TA was added with OA, it did not showed additive increasing effects rather than partial attenuation of OA-stimulated cAMP production by 30% decreasing.

**Keywords:** cAMP, biogenic amines, Two-spotted mites, modulation

## 1. Introduction

Much interest has been drawn on the bioamines receptors (for instance, octopamine, tyramine, dopamine etc.) and their pharmacological profiles in insects and some mites [1, 2]. In comparison with insects, mites may have different bioamines receptor subtypes and hence are of scientific interest [3, 4]. Knowles et al. found that bioamines were present in bulb mites tissues [5], yet the simulation or attenuation behaviours of various bioamines on the cAMP production of mites are still not clear. Matsumura et al. reported [6] the influence of several pesticide including chlordimeform and its metabolite on the cAMP levels of two-spotted mites. They concluded that the agonist effects of formamidine acaricides were expressed primarily through the OA-sensitive adenylate cyclase. In this paper, the interaction of biogenic amines including octopamine, dopamine, tyramine, serotonin, noradrenaline with octopamine / tyramine receptors and the resulting stimulation or attenuation effects of cyclic AMP production in vitro were investigated in the membrane homogenates of two-spotted spider mite, *Tetranychus cinnabarinus*. Interestingly, we found both stimulating and attenuation effects of different bioamines on the cAMP production.

## 2. Methods

Whole mites were homogenized in 10mM phosphate (containing 0.25M sucrose) buffer (1200 rpm, 10 strokes, 0℃). The homogenate was centrifuged at 900g for 10min and the resulting supernatant was centrifuged at 9000g for 15min. The 9000g pellets were re-suspended by hand in incubation buffer (80mM Tris-maleate pH 7.4, EGTA 0.5mM, 2-mercaptoethanol 1mM and $MgCl_2$ 8 mM) for 15 min. The protein concentration was determined by Bradford method. The adenylate cyclase assay was achieved by mixing re-suspended membrane 100μL, theophyline (40μl 0.5mM), GTP (20μL 1mM), and octopamine or compounds solution (20μL). After pre-incubation at 30℃ for 15min., 20μL ATP was added to initiate the enzyme reaction. After 5 minutes the reaction was stopped by heating 2min at 90℃ bath. cAMP was determined by the standard binding protein assay system with modifications [6].

## 3. Results and Discussion

OA, TA and DA agonists and antagonists were utilized to test the characteristics of the positive or negative modulation of cAMP production. Figure 1 showed a typical cAMP standard curve determination under RIA method. It's found that 100μM OA could cause around 310~420% increasing of cAMP comparing with the basal treatment, which was done with 10μm theophyline (as shown in Figure 2, IC50=7.6μM). The stimulation effect of OA could be inhibited completely by OA antagonist, like 100μM miaserin. The adenylate cyclase (AC) system could be activated by 10μM forskolin with an average stimulation of around 1200% enhancement of cAMP production. 5-HT and NA displayed no significant potential stimulating or attenuation effects on the AC activity. It's very interesting to find that DA, which has similar structure with octopamine except its m-hydroxyl group in the phenyl ring, could significantly inhibit the cAMP production at above 10μM concentration (Table 1). Further study showed that $IC_{50}$ of DA as an antagonist behaviour was 25.1μM (as shown in Figure 3). It's apparent that 1mM DA could attenuate cAMP stimulation to a level below the basal. The attenuation of DA could be partially recovered from TA or DA antagonist yohimbine. More interestingly, with 10μM TA alone, it could cause about 40% increasing of cAMP. While 100μM TA was added with OA, it did not showed additive increasing effects rather than partial attenuation of OA-stimulated cAMP production by 30% decreasing. This phenomenon could not be explained similarly in *Bombyx mori* [7], which was supposed to be positively and negatively modulated by 5-phenyloxazoles. Clonidine and talazoline, two medicals with DA agonist activity, have no attenuation or stimulating effects on cAMP production of mites.

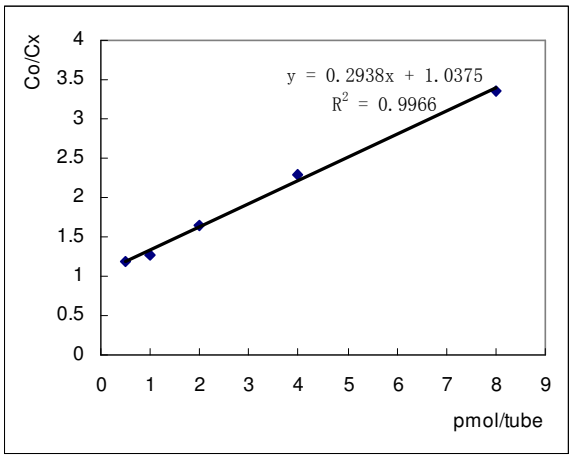


Figure 1. A typical standard curve for cAMP quantification by the RIA method (C0: Blank cpm, Cx: average cpm of sample, each point was tested by two duplicates, cpm values were means of two measures in 5 minutes count).

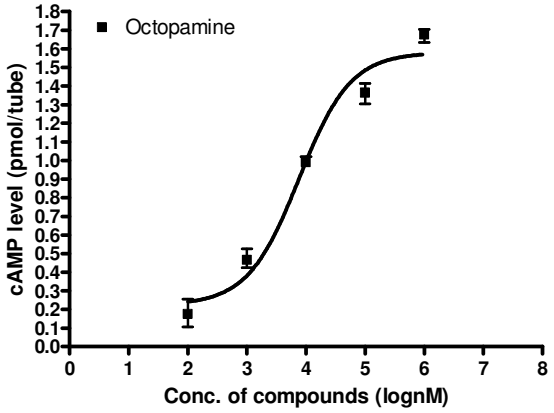


Figure 2. Dose-response curve of OA-stimulated cAMP production in two-spotted mites membranes (IC50: 7.6μM, SD=0.22). X axis: various concentrations of octopamine in 0.5% DMSO; Y axis: Total stimulated cAMP levels minus that of basal, in pmol/tube (basal=1.85 pmol/tube), Y error bars stand for the error of the means (n=3).

Table 1 cAMP levels in two-spotted mites membranes under various chemical stimulations

| Compounds Name | Concentration (μM) | cAMP level (in average ±SD, n=3) (pmol/tube) |
|---|---|---|
| Basal (0.5% DMSO) | | 1.85 ± 0.31 |
| Octopamine | 200 | 4.04 ±0.15 |
| Tyramine | 200 | 2.55 ± 0.28 |
| Tyramine + Octopamine | 200 + 200 | 3.17 ± 0.17 |
| Dopamine | 200 | 0.01 ± 0.07 |
| Dopamine + Octopamine | 200+ 200 | 0.33 ±0.13 |
| Forskolin | 100 | 7.01 ± 0.24 |
| Dopamine + Forskolin | 200 + 100 | 5.34 ±0.30 |
| Dopamine + Yohimbine + Octopamine | 200+ 10 + 200 | 2.55 ±0.29 |
| Clonidine | 200 | 1.72 ±0.18 |
| Clonidine + Octopamine | 200 + 200 | 4.23 ±0.17 |
| Tolazoline | 200 | 1.86 ±0.22 |
| Tolazoline + Octopamine | 200 + 200 | 4.17 ±0.24 |
| Serotonin | 200 | 1.78 ±0.17 |
| Serotonin + Octopamine | 200 + 200 | 4.04 ±0.21 |
| Noradrenaline | 200 | 1.79 ±0.23 |
| Noradrenaline + Octopamine | 200 + 200 | 4.36 ±0.36 |

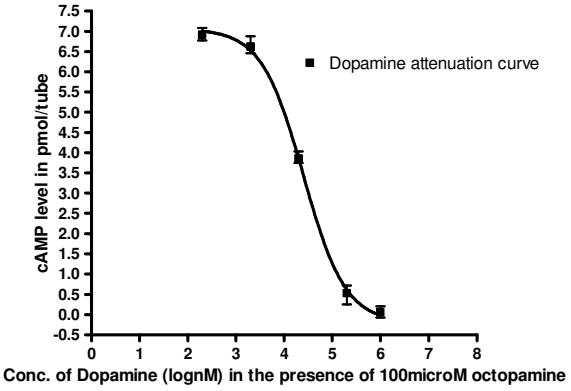


Figure 3 Dose-response curve of DA-attenuated cAMP production in two-spotted mites membranes, EC50=25.1μM, SD=0.14. (X axis: various concentrations of Dopamine in 0.5% DMSO; Y axis: cAMP levels, in pmol/tube )

With the cAMP function assay method, we tested the effects of various bioamines on the two-spotted mite membranes. Binding assays with octopamine, dopamine, and tyramine labelled ligands (detailed experimental procedure and results no shown) showed that no significant specific binding was found, indicating that a trace level receptor may be present.

## 4 Conclusions

The above experimental results imply that an octopamine/tyramine receptor may be present rich in the two-spotted spider mite, *Tetranychus cinnabarinus*. A hypothesis model of the bioamines receptor system was established with the receptor linked to the AC system with a bi-modulation mode by OA or TA mimics. We may propose that TA may bind with both sites, while OA or DA could selectively bind the OA or TA site respectively, with the latter leading to the attenuation effect of cAMP production.

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