Neuromodulatory Effects of Octopamine Agonists at Neuromuscular Junctions in the Mealworm, *Tenebrio molitor* L. (Coleoptera: Tenebrionidae)

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The modulatory effects of biogenic amines related to octopamine were studied at the neuromuscular junctions of larvae of the mealworm, *Tenebrio molitor*. Intracellular recordings were made with glass microelectrodes from ventral longitudinal muscle fibers. Phenolamines (octopamine, synephrine) and catecholamines (noradrenaline, adrenaline) reversibly potentiated the amplitude of excitatory junctional potentials (EJPs), but gave no effect either in the resting membrane potentials or on the time constant of the decay phase of the EJPs. EJPs were potentiated to a greater extent by the phenolamines than by the catecholamines. Potentiation was antagonized by phentolamine. These results suggest some structural specificity in the insect octopaminergic system.

Key words: octopamine, phenolamines, catecholamines, neuromuscular junction, *Tenebrio molitor*

INTRODUCTION

It is widely accepted that octopamine is an important biogenic amine in insects (EVANS, 1985). It has been shown to function as a neurotransmitter, a neurohormone and a neuromodulator (EVANS, 1980, 1985; ORCHARD, 1982). Its modulatory action has been studied in a neuromuscular system (EVANS and O'SHEA, 1978; KLAASSEN and KAMMER, 1985; O'SHEA and EVANS, 1979). At synapses between motor neurons and somatic muscles (neuromuscular junctions), L-glutamate is assumed to be an excitatory transmitter (NISTRI and CONSTANTI, 1979; USHERWOOD, 1978), and this neuromuscular transmission is enhanced by octopamine in the locust (O'SHEA and EVANS, 1979), *Manduca* (KLAASSEN and KAMMER, 1985) and the mealworm (HIDOHI and FUKAMI, 1987).

The enhancement of neuromuscular transmission that potentiates the amplitude of EJPs in the mealworm is attributed to presynaptic increase in the release of neurotransmitter (HIDOHI and FUKAMI, 1987). We shall describe the effects of biogenic amines that are structural analogues of octopamine (Table 1).

MATERIALS AND METHODS

Second or third instar larvae of the mealworm, *Tenebrio molitor*, were used throughout the experiment. The ventral longitudinal muscle fibers of abdominal segments were exposed by slitting open the dorsal midline and removing the viscera and fat

bodies. Each dissected insect was mounted on a perfusion chamber made of sylgard lesine and was continuously perfused with *Tenebrio* saline containing 70 mM NaCl, 30 mM KCl, 14.4 mM MgCl₂, 0.6 mM CaCl₂, 445 mM glucose and 5 mM HEPES. The pH of this saline was adjusted to 7.2 with NaOH. All chemicals were directly dissolved in the saline and applied by perfusate (tyrosine purchased from Nakarai Chemical Co., phentolamine mesylate from Ciba-Gigy Co., and all other amines from Sigma Chemical Co.). Each amine was obtained as the D,L-isomer.

Intracellular recordings from muscle fibers were made by a glass microelectrode filled with 3 M KCl, of which resistance was 5 to 10 MΩ. The motor nerve innervating the ventral muscles was stimulated at a juxtaposition to the segmental ganglion with square voltage pulses of 0.1 ms duration by a pair of Ag-hook electrodes so as to elicit EJPs. Muscle fibers, of which resting potentials were between -40 and -50 mV and of which EJP amplitude was smaller than 10 mV, were used for the experiments. EJPs obtained from a muscle fiber were normalized in the following manner; 16 EJPs evoked at 2 Hz just before the perfusion with amine (control) and 3 min after the perfusion (treated) were averaged by means of the signal averager (Nihon-kohden, DAT-1100) in order to improve signal-noise ratio.

All experiments were carried out at room temperature (24–26°C).

RESULTS

Phenolamines, e.g., octopamine and synephrine, enhanced neuromuscular transmission, potentiating the amplitude of EJPs at the concentrations of higher than 10⁻⁸ M, whereas catecholamines, e.g., noradrenaline and adrenaline, did so at the concentrations higher than 10⁻⁵ M (Fig. 1 and 2). The potentiating actions of these four amines were reversible. Potentiated EJPs recovered within 20 min after being perfused with normal saline. Dose-response relationships are shown in Fig. 2. The two phenolamines potentiated EJPs in an identical and dose-dependent manner. The two catecholamines potentiated EJPs in the same manner as did the phenolamines. The potentiation of the phenolamines, however, was stronger than that of the catecholamines. Tyramine

Table 1. Structures of octopamine-related compounds tested in this study and their modulatory potency.

Compound ^a		Potency ^b	
Tyrosine	H	CH ₂ CH(COOH)NH ₂	—
Tyramine	H	CH ₂ CH ₂ NH ₂	—
Octopamine	H	CH(OH)CH ₂ NH ₂	++
Synephrine	H	CH(OH)CH ₂ NHCH ₃	++
Noradrenaline	OH	CH(OH)CH ₂ NH ₂	+
Adrenaline	OH	CH(OH)CH ₂ NHCH ₃	+

^a Structure of R₁ is represented in second column, and R₂ is in third column.

^b Modulatory potency on EJP amplitude.

—: no effect; +: effective; ++: more effective.

Fig. 1. The potentiating action on EJPs of phenolamines and catecholamines. Each EJP was averaged from 16 EJPs evoked at 2 Hz just before perfusion with amines (control) and 3 min after the perfusion (treated). Octopamine (10^{-6} M), synephrine (10^{-6} M), noradrenaline (10^{-5} M) or adrenaline (10^{-5} M) were perfused. Calibration: 5 mV, 30 ms.

Fig. 2. Dose-response relationship of amines to the amplitude of EJPs. Each point represents the mean of differences (treated minus control) obtained from 7–14 determinations. Vertical bars: S.E.

and tyrosine caused little potentiation up to 10^{-4} M. These results are also summarized in Table 1.

The resting membrane potentials of muscle fibers were slightly hyperpolarized by higher doses of octopamine (10^{-5} M), synephrine (10^{-5} M) and tyrosine (10^{-4} M). These amines, as well as tyrosine at lower concentrations, and the other amines had no effect on the resting membrane potentials of muscle fibers (Fig. 3).

The time constants estimated from a single exponential decay phase of EJPs were unchanged. The time constants (ms) of 10^{-5} M treatments were as follows (mean $\pm$ S.E. from 7–13 determinations, control/treated); octopamine ($21.2 \pm 1.6/21.5 \pm 1.6$), synephrine ($19.6 \pm 1.6/19.6 \pm 2.1$), noradrenaline ($21.3 \pm 1.3/20.0 \pm 1.2$), adrenaline ($20.3 \pm 1.3/19.6 \pm 1.6$), tyramine (20.6 ± 1.7), tyrosine (20.6 ± 1.9). These values or those obtained at other concentrations were not significant against controls by paired sample *t*-test ($p > 0.1$).

The antagonistic action of phentolamine, an α -adrenergic blocker, showed that the potentiations of octopamine, synephrine, noradrenaline and adrenaline were competitively antagonized by phentolamine (Fig. 4). At 10^{-5} M, phentolamine produced a near-parallel rightward shift in their dose-response curves with no significant change in the maximal attainable response ($p > 0.1$, two sample *t*-test).

The dose-inhibition relationship of phentolamine on octopamine- and noradrenaline-induced potentiations were compared. Concentrations of octopamine and noradrenaline were determined so that the potency was made even. The appropriate concentrations were 3×10^{-6} M for octopamine and 3×10^{-5} M for noradrenaline, making the potentiating amplitudes reach 3.5 mV. Octopamine- and noradrenaline-induced potentiations were antagonized by phentolamine in the same manner (Fig. 5). At 10^{-5} M, antagonistic actions on the two amines were not significant ($p > 0.2$, two sample *t*-test). ID_{50} (50% inhibition dose) was estimated at 1.3×10^{-6} M for both amines.

Fig. 3. Dose-response relationship of amines to the resting membrane potentials. Each point represents the mean of differences ($N=7-14$). Vertical bars: S.E. Symbols are the same as in Fig. 2.

Fig. 4. Antagonistic action of phentolamine on amine-induced potentiation. Each point represents the mean of differences ($N=6-7$) in the presence of phentolamine (10^{-5} M). Vertical bars represent S.E. of the mean. Results with octopamine and synephrine correspond to ordinate on the left, and results with noradrenaline and adrenaline correspond to ordinate on the right. Open symbols and dotted lines were redrawn from Fig. 2 for comparison.

Fig. 5. Dose-response relationship of the antagonistic action of phentolamine to the amine-induced potentiation. Each point represents the mean of percent inhibition obtained from 6 or 7 determinations. Vertical bars. S.E.

DISCUSSION

Octopamine and three related monoamines, synephrine, noradrenaline and adrenaline, modulated neuromuscular transmission. Dose-response relationships and antagonistic action of phentolamine indicate that these amines act on the same site. Quantum contents estimated by the extracellular EJP failures are greatly increased

by octopamine, which potentiated EJP amplitude (HIDOH and FUKAMI, 1987). This result suggests that the release of presynaptic transmitter is enhanced by octopamine at the neuromuscular junction in *T. molitor*. It is also considered that the other three amines act on presynaptic terminals in the same manner as octopamine. Presynaptic actions of these amines are further supported by a slight effect on the resting membrane potentials and by the absence of any effect on the time constant of EJP decay phase.

The amplitude of EJPs was maximally potentiated by phenolamines rather than by catecholamines in *T. molitor*. In locust extensor tibiae muscle, phenolamines are more effective than catecholamines in potentiating twitch tension (O'SHEA and EVANS, 1979) and in slowing myogenic rhythm (EVANS and O'SHEA, 1978). In *Manduca*, the maximum following frequency (MFF) at which the muscle contracts in response to train stimuli is increased to a greater extent by phenolamines than by catecholamines (KLAASSEN and KAMMER, 1985). It is generally considered that phenolamines are more effective than catecholamines in affecting insect octopaminergic systems.

The interpretation concerning the potency of phenolamines is complicated. In broken cell preparations of cockroach brain (HARMER and HORN, 1977) and firefly lanterns (NATHANSON, 1979), octopamine was more potent in increasing the activity of adenylate cyclase than synephrine. In the neuromuscular system of *Manduca*, octopamine was also more potent in increasing the MFF than synephrine (KLAASSEN and KAMMER, 1985). In contrast, synephrine was more potent than octopamine in inducing luminescence in the light organ of the firefly (CARLSON, 1968), and potentiating twitch tension (O'SHEA and EVANS, 1979) and slowing the myogenic rhythm (EVANS and O'SHEA, 1978) in the locust. Our results showed equivalent potentiation by octopamine and synephrine. Pharmacological effects often depend on receptor subtypes existing in different species and organs. Different drugs may have differential access to target sites or may be inactivated at different rates (EVANS, 1980). EVANS (1981) has suggested that inactivation by a high-affinity uptake mechanism is unlikely to be interfering with pharmacological profiles. The reason why phenolamines give different efficacy in different preparations remains unknown in insect nervous tissues.

In terms of structure specificity, monoamine with a single hydroxyl on the aromatic ring is essential. It also seemed that the β -hydroxy side chain is required for modulatory action. The structure specificity of our results supports the importance of octopamine as a naturally-occurring amine in the insect nervous system.

REFERENCES

- CARLSON, A. D. (1968) Effect of adrenergic drugs on the lantern of larval *Photuris* firefly. *J. Exp. Biol.* **48**: 381–387.
- EVANS, P. D. (1980) Biogenic amines in the nervous system. *Adv. Insect Physiol.* **15**: 317–473.
- EVANS, P. D. (1981) Multiple receptor types for octopamine in the locust. *J. Physiol. Lond.* **318**: 99–122.
- EVANS, P. D. (1985) Octopamine. In *Comprehensive Insect Physiology Biochemistry and Pharmacology*. Vol. 11. (G. A. KERKUT and L. I. GILBERT, eds.). Pergamon Press Ltd., Oxford, p. 499–530.
- EVANS, P. D. and M. O'SHEA (1978) The identification of an octopaminergic neurone and modulation of a myogenic rhythm in the locust. *J. Exp. Biol.* **73**: 235–260.
- HARMER, A. J. and A. S. HORN (1977) Octopamine-sensitive adenylate cyclase in cockroach brain: effects of agonists, antagonists and guanylyl nucleotides. *Mol. Pharmacol.* **13**: 512–520.
- HIDOH, O. and J. FUKAMI (1987) Presynaptic modulation by octopamine at a single neuromuscular junction in the mealworm (*Tenebrio molitor*). *J. Neurobiol.* **18**: 315–326.

- KLAASSEN, L. W. and A. E. KAMMER (1985) Octopamine enhances neuromuscular transmission in developing and adult moths, *Manduca sexta*. *J. Neurobiol.* **16**: 227-243.
- NATHANSON, J. A. (1979) Octopamine receptors, adenosine 3', 5'-monophosphate, and neural control of firefly flashing. *Science* **203**: 65-68.
- NISTRI, A. and A. CONSTANTINI (1979) Pharmacological characterization of different types of GABA and glutamate receptors in vertebrate and invertebrate. *Prog. Neurobiol.* **13**: 117-235.
- ORCHARD, I. (1982) Octopamine in insects: neurotransmitter, neurohormone and neuromodulator. *Can. J. Zool.* **60**: 659-669.
- O'SHEA, M. and P. D. EVANS (1979) Potentiation of neuromuscular transmission by an octopaminergic neurone in the locust. *J. Exp. Biol.* **79**: 169-190.
- USHERWOOD, P. N. R. (1978) Amino acids as neurotransmitters. *Adv. Comp. Physiol. Biochem.* **7**: 227-310.