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Calcium involvement in mediating the action of octopamine and hypertrehalosemic peptides on insect haemocytes

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Octopamine, the hypertrehalosemic peptides HT-I and HT-II and the calcium ionophore A23187 elevate intracellular calcium levels in cultured haemocytes of *Malacosoma disstria* (Lepidoptera: Lasiocampidae). The octopamine-mediated response is dose-dependent and the magnitude of the response is influenced by the concentration of calcium in the incubation medium. Mianserin, an inhibitor of octopamine-mediated elevation of cyclic AMP production, blocks the octopamine-mediated increase in intracellular calcium but has no effect on the HT-I- and HT-II-mediated responses. The results indicate that some effects of octopamine are mediated through agonist-dependent calcium gating whereas others are expressed through receptors coupled to adenylate cyclase. The study also confirms previous suggestions that HT-I and HT-II elevate intracellular calcium concentrations.

Octopamine; Hypertrehalosemic peptide; Ca^{2+}; (Insect, Hemocyte)

1. INTRODUCTION

Octopamine is an important neurotransmitter, neurohormone and neuromodulator in insects [1]. Extensive studies on a variety of tissues from several insect species indicate that some actions of octopamine are mediaed through the interaction of octopamine with a specific receptor that is coupled to an adenylate cyclase complex and consequent elevation of intracellular concentrations of cyclic AMP [2–7]. At least two classes of octopamine receptor have been recognised pharmacologically and it is likely that, as is the case with vertebrate catecholamine, there are other mechanisms by which octopamine action may be mediated. An obvious possibility in this regard involves agonist-dependent calcium gating, similar to that described for the action of 5-hydroxytryptamine on salivary glands of adult *Calliphora* [8]. The present study was undertaken with the primary objective of demonstrating octopamine-induced elevation of intracellular calcium levels in insect cells.

The hypertrehalosemic peptides (HT-I and HT-II) are functionally similar to vertebrate glucagon in that they activate glycogen phosphorylase in insect fat body to cause marked elevation of trehalose, the major haemolymph sugar of insects [9]. However, the insect peptides, unlike glucagon, do not mediate their effects through production of cyclic AMP and, instead, may induce increased intracellular concentrations of calcium. Therefore, the effects of HT-I and HT-II on intracellular calcium levels in insect cells were also investigated in the current study.

2. MATERIALS AND METHODS

Cells were taken from a haemocyte cell line derived from *Malacosoma disstria* (Lepidoptera: Lasiocampidae) and maintained in this laboratory under the conditions in [10]. Changes in cytosolic calcium concentrations were observed by

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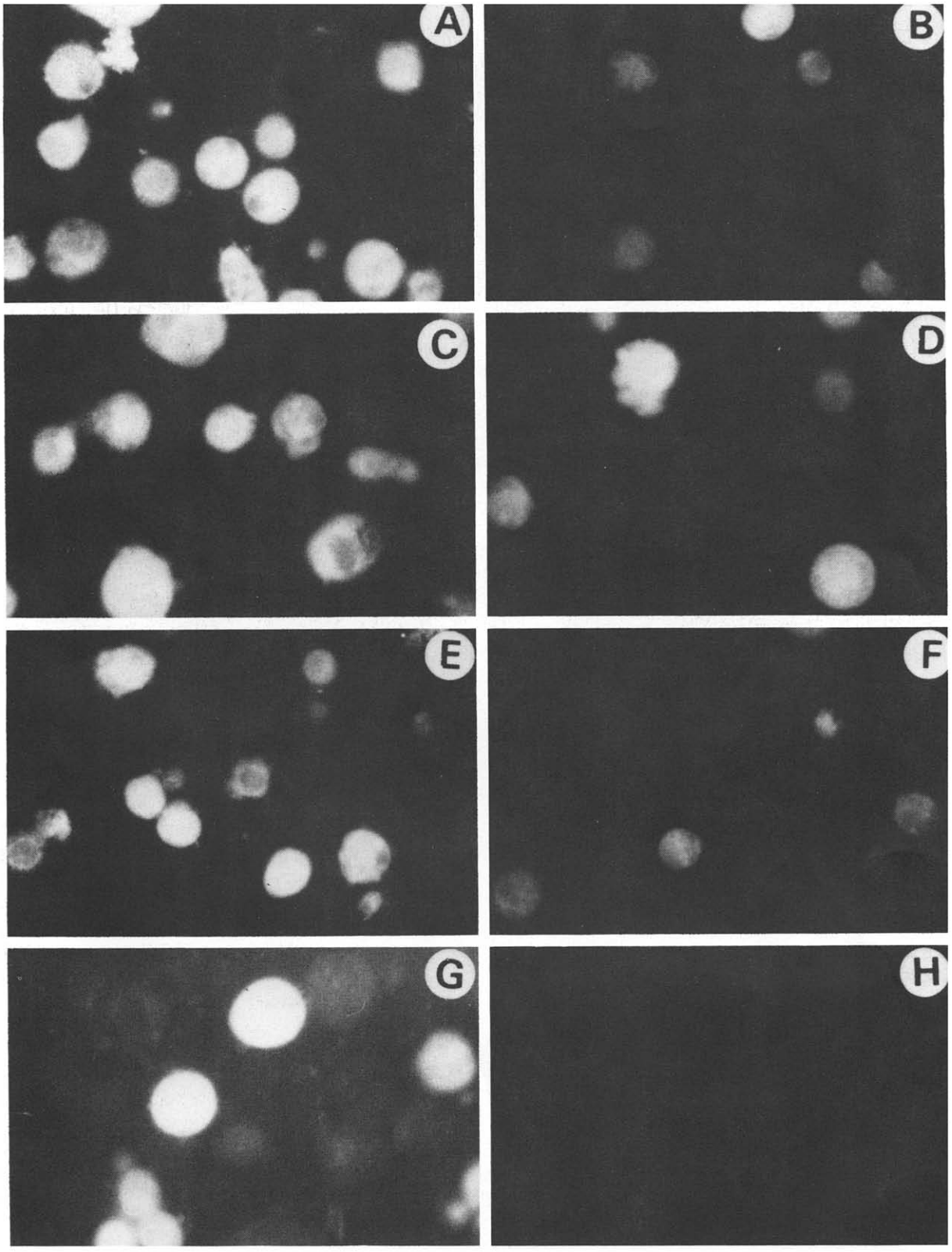
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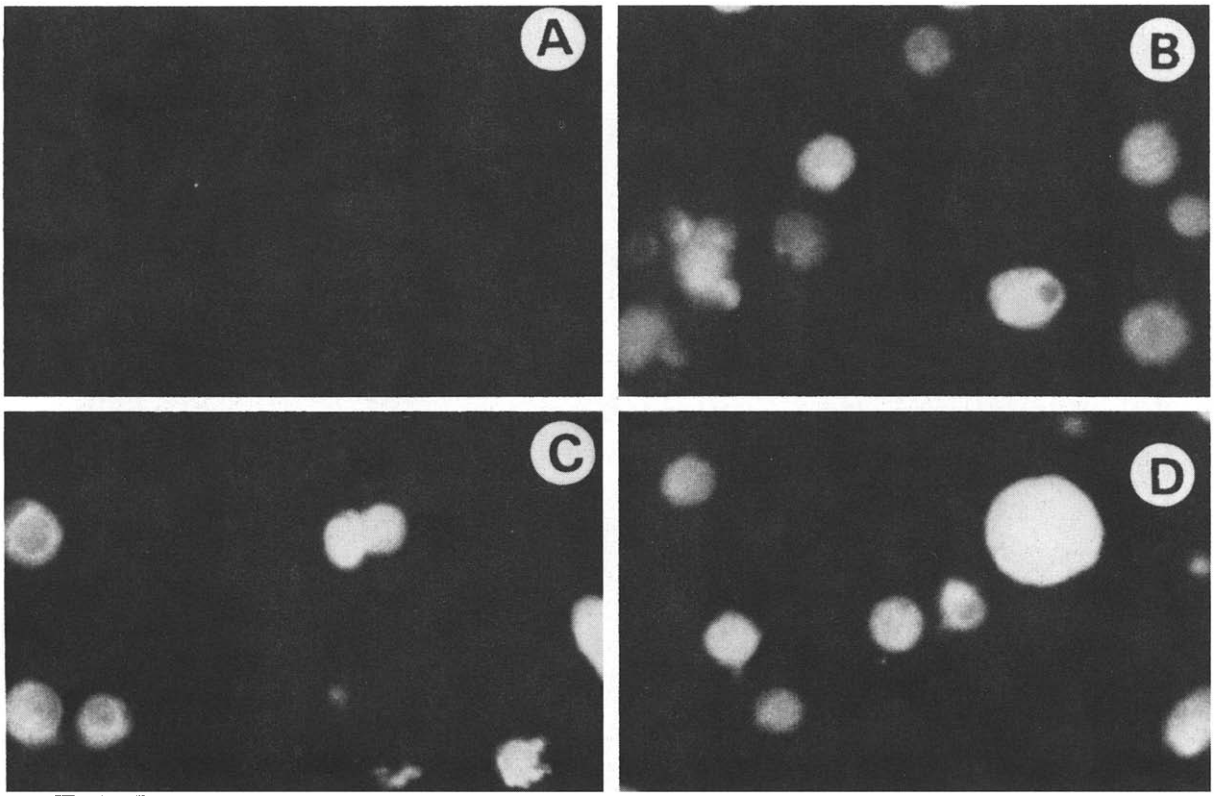

Fig.2. Effect of mianserin, in the presence of Ca^{2+}, on octopamine-, HT-I-, HT-II- and A23187-mediated elevation of intracellular calcium levels in cultured haemocytes of *M. disstria*. Experimental details are described in section 2. (A) Octopamine, (B) HT-I, (C) HT-II, (D) A23187.

fluorescence microscopy using the calcium-sensitive dye, fura 2AM [11].

The cells were sedimented by low speed centrifugation (200 × *g*), washed twice with saline and then resuspended at a concentration of approx. 10^6 cells/ml saline. Following a 10-min incubation in 1 μM fura 2AM, the cells were sedimented to remove excess dye and resuspended in saline. The pre-loaded cells were incubated in normal (contains 1.8 mM Ca^{2+}) or calcium-free insect saline [12] and the test compound(s) added to yield a final concentration of 1 μM. After mixing, the cells were viewed under a Nikon Optiphot at 200 × magnification using a 'U' filter module. Photographs were recorded on FujiChrome 400 ASA film.

Intracellular calcium concentrations were determined using Arsenazo-III [13]. A 400 μl aliquot of cell suspension was incubated with 40 μl test compound at 25°C for 10 min. Following incubation, the suspension was placed in crushed ice (0–4°C) before being centrifuged at 200 × *g* and the sedimented cells washed with calcium-free saline. The washed cells were then homogenised, centrifuged at 500 × *g* and the supernatant used for determination of calcium concentration.

Octopamine, Arsenazo-III and A23187 were obtained from Sigma, St. Louis. The hyper-

Fig.1. Effect of octopamine, HT-I, HT-II and A23187, in the presence (A,C,E) and absence (B,D,F) of calcium, on intracellular calcium levels of cultured haemocytes of *M. disstria*. Experimental details are described in section 2. (A,B) Octopamine, (C,D) HT-I, (E,F) HT-II, (G) A23187, (H) control + Ca^{2+}.

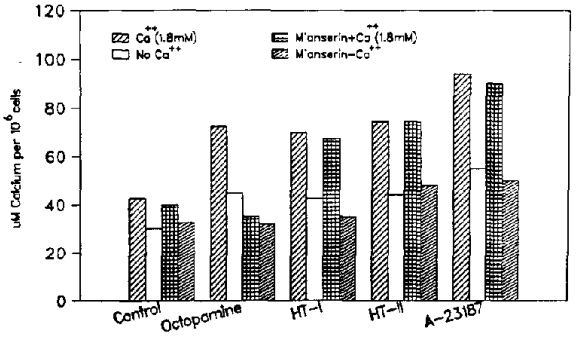

Fig.3. Effect of octopamine, HT-I, HT-II and A23187 on intracellular calcium concentrations in cultured haemocytes of *M. disstria*. Standard deviations for all values are less than 10% ($n = 4$).

trehalosemic peptides were generously donated by Dr Robert Scarborough and the Zoecon Corp., Palo Alto, CA.

3. RESULTS

Octopamine, the hypertrehalosemic peptides HT-I and HT-II and the calcium ionophore A23187 cause a marked increase in the fluorescent intensity of cells incubated in normal and calcium-free saline compared with untreated cells (fig. 1a–f). Mianserin, the antagonist of octopamine-mediated elevation of cyclic AMP in insects, inhibits the octopamine effect on intracellular calcium levels but has no effect on the actions of HT-I, HT-II or A23187 (fig.2a–d).

The results obtained with fura 2AM were confirmed by estimating intracellular calcium concentrations in cells exposed to the various treatments. The data indicate that octopamine, HT-I and HT-II increase intracellular calcium concentrations by approx. 70% in normal insect saline and by 40–50% in calcium-free saline (fig.3). Furthermore, the octopamine-mediated increases are inhibited by mianserin whereas the elevated calcium levels resulting from treatment with HT-I, HT-II and A23187 are unaffected by the drug (fig.3).

The octopamine-mediated elevation of intracellular calcium levels is dose-dependent with respect to octopamine and calcium (table 1). At high calcium concentrations (3.6 mM), 5×10^{-6} M octopamine causes a 4-fold increase in intracellular calcium whereas this concentration of octopamine elicits a 2.44-fold increase at normal calcium concentrations (1.8 mM). In calcium-free medium, 5×10^{-6} M octopamine increases intracellular calcium levels by 2.16-fold.

4. DISCUSSION

Octopamine-sensitive receptors that are coupled to adenylate cyclase complexes have been demonstrated in several insect tissues and species [2–7]. Octopamine increases cyclic AMP production in cultured haemocytes of *M. disstria* [14] and, in addition, elicits rapid elevation of cytosolic

Table 1

Effect of varying concentrations of octopamine and added calcium on cytosolic calcium concentrations in cultured haemocytes of *M. disstria*

Concentration of octopamine (μM)	Cytosolic calcium concentration (μM/10^6 cells)		
	No added Ca^{2+}	1.8 mM Ca^{2+}	3.6 mM Ca^{2+}
0	26.40 ± 3.81	27.44 ± 3.00	36.24 ± 3.77
0.5	33.00 ± 2.29	48.87 ± 2.50^{ab}	51.95 ± 5.04^{ab}
1.0	40.98 ± 4.51^{a}	63.16 ± 4.67^{ab}	67.84 ± 3.87^{ab}
2.0	53.51 ± 4.54^{a}	64.68 ± 3.26^{abc}	87.49 ± 4.76^{abc}
5.0	56.20 ± 1.40^{a}	67.02 ± 1.91^{abc}	151.55 ± 3.41^{abc}

Values indicate mean ± SE for 3 determinations. Statistical significance at $p < 0.05$ is indicated when values differ from controls with no added octopamine[a], no added extracellular calcium[b] and when different concentrations of extracellular calcium are added[c]

calcium concentrations (figs 1 and 3). Octopamine-induced increases in cytosolic calcium occur in haemocytes that are maintained in calcium-free medium (table 1) thus suggesting that the mechanism of octopamine action involves mobilisation of intracellular calcium reserves. However, the magnitude of the increase in cytosolic calcium levels is dependent upon the concentration of extracellular calcium (table 1), therefore, the mechanism may also involve stimulation of calcium entry from the extracellular environment. 5-Hydroxytryptamine elicits increased production of inositol-1,4,5-triphosphate (IP_3) in plasma membrane preparations of blowfly salivary glands through interaction with a receptor that is coupled to phospholipase C by a G protein [15]. IP_3 causes release of calcium from intracellular pools [16] and, either directly or indirectly, elicits the opening of 'calcium gates' in the plasma membrane [17]. It is possible that the octopamine-receptor, which is involved in the increased cytosolic calcium concentrations observed in the present study, may also be coupled to phospholipase C.

Mianserin, which is an inhibitor of 5-hydroxytryptamine- and histamine-receptors in vertebrate systems, is a potent inhibitor of octopamine-mediated activation of adenylate cyclase in insects [2,18]. Mianserin also blocks the octopamine-induced elevation of cytosolic calcium levels in cultured haemocytes (figs 2 and 3) but does not affect the actions of HT-I, HT-II or A23187 in this regard. Thus, the octopamine-mediated effect is expressed through a different receptor than that responsible for mediating the actions of the hypertrehalosemic hormones.

Figs 1 and 3 demonstrate also that the hypertrehalosemic hormones, HT-I and HT-II, elevate intracellular concentrations of calcium and, therefore, confirm previous suggestions [9,12] that these peptides bind to a receptor to induce increased levels of cytosolic calcium. Vertebrate glucagon is now believed to possess two distinct receptors, one of which is coupled to inositol phospholipid breakdown and calcium gating and the other to the adenylate cyclase complex [14]. Thus, the hypertrehalosemic peptides may function in an analogous manner to those actions of glucagon that are mediated through intracellular calcium.

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