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Short Communication

Dopamine Stimulates Production of Cyclic AMP by the Salivary Gland of the Cockroach, *Nauphoeta cinerea*

C. W. Grewe[1] and J. W. Kebabian[1,2]

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SUMMARY

1. Dopamine increases cAMP production in the salivary gland of *Nauphoeta cinerea.*

2. This effect is mimicked by 6,7-ADTN and blocked by fluphenazine.

3. Although dopamine is a neurotransmitter in this tissue and elicits fluid secretion and hyperpolarization, the physiological significance of the biochemical effect of dopamine is unknown.

Dopamine is a neurotransmitter in the salivary gland of *Nauphoeta cinerea,* and several physiological effects of dopamine upon this tissue (hyperpolarization and enhanced fluid secretion) have been characterized [see House (1980) for a review and references]. The physiological and pharmacological evidence for the existence of a dopamine receptor in *N. cinerea* salivary gland together with the previous demonstration of a dopamine-sensitive adenylate cyclase in other insect tissues (Harmar and Horn, 1977) prompted this investigation of the dopamine-sensitive adenylate cyclase in *N. cinerea* salivary gland.

Alone, dopamine caused virtually no accumulation of cyclic AMP in *N. cinerea* salivary gland tissue. In the presence of 3-isobutyl-1-methylxanthine (IBMX), a phosphodiesterase inhibitor, dopamine caused a substantial accumulation of cAMP (Fig. 1, left). In the presence of 1 m*M* IBMX, the maximal response to dopamine was a

[1] Experimental Therapeutics Branch, NINCDS, NIH, Bethesda, Maryland 20205.
[2] To whom correspondence should be addressed at 10/6D-16, NIH, Bethesda, Maryland 20205.

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5-fold increase in the level of cAMP; half-maximal cAMP accumulation occurred in the presence of 10 μM dopamine (Fig. 1, right). In addition, dopamine substantially increased adenylate cyclase activity in a cell-free homogenate of *N. cinerea* salivary gland (Fig. 2, left). Furthermore, 2-amino-6,7-dihydroxy-1,2,3,4,-tetrahydronaphthalene (ADTN; Miller *et al.*, 1974) mimicked this stimulatory effect of dopamine. Half-maximal stimulation of enzyme activity was achieved with 6×10^{-7} M dopamine and 4×10^{-6} M ADTN, respectively. Maximal stimulation of adenylate cyclase activity achieved with either agent was approximately the same; the maximally stimulatory effects of dopamine and ADTN were nonadditive (Fig. 2, left). The ability

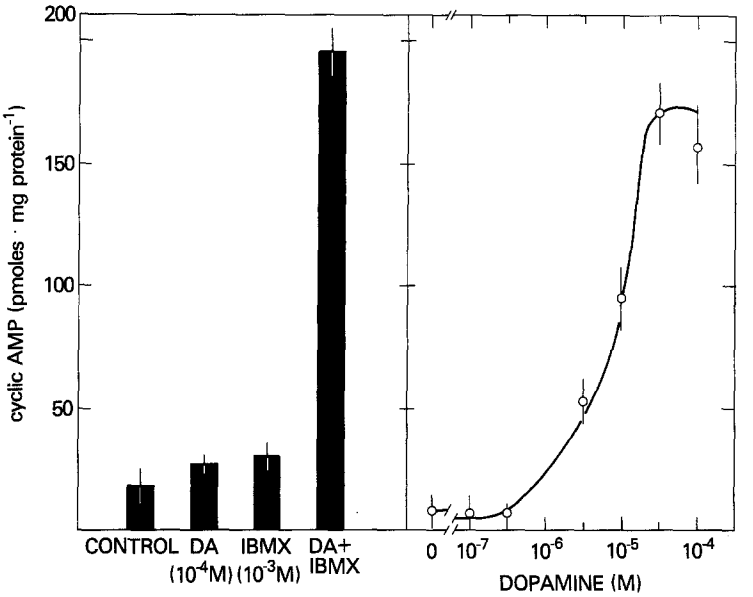

Fig. 1. Dopamine stimulates cAMP accumulation in intact salivary gland of *N. cinerea*. Left: *N. cinerea* salivary gland tissue was incubated in the presence of no drug (control), 100 μM dopamine (DA); 1 mM 3-isobutyl-1-methylxanthine (IBMX), or a combination of dopamine and IBMX (DA + IBMX). Right: *N. cinerea* salivary gland tissue was incubated in the presence of 1 mM IBMX and the indicated concentrations of dopamine. *Nauphoeta cinerea* were maintained on a diet of rat chow and water. Salivary glands were removed and the grape-like clusters of acinar cells were carefully teased off the ducts. The acinar tissue was acutely stored in a solution containing 160 mM NaCl, 20 mM glucose, 10 mM KCl, 5 mM $CaCl_2$, 5 mM Tris, and 4 mM HCl (pH 7.6). After the tissue was collected from 10 to 15 animals, it was washed, teased apart, and resuspended (5 pairs of glands/ml) in this solution. Aliquots of the tissue (60 μl) were transferred to 50 μl of the salt solution containing the indicated concentrations of drugs. The tissue was incubated for 10 min at 35°C and then the incubation tubes were boiled for 2 min. Tubes were centrifuged and the supernatant fluid was separated from the particulate material. The pH of the supernatant fluid was adjusted to pH 5.5 and cAMP was determined by the method of Brown *et al.* (1971) with previously described modifications (Munemura *et al.*, 1980). Protein content of the particulate material was determined by the method of Lowry *et al.* (1951). Data present the amount of cAMP in each sample normalized for the protein content of each sample and represent the mean ± SE ($N = 4$).

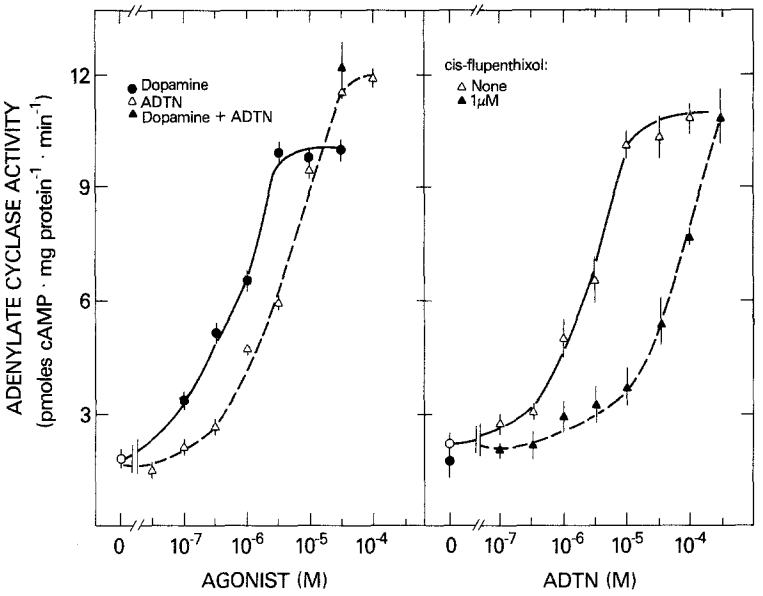

Fig. 2. Dopamine and ADTN stimulate adenylate cyclase activity in the salivary gland of *N. cinerea*. Left: Adenylate cyclase activity was determined in the presence of the indicated concentrations of dopamine (filled circles), ADTN (open triangles), or a combination of dopamine and ADTN (each at a concentration of 30 μM; filled triangle). Right: Adenylate cyclase activity was determined in the presence of the indicated concentrations of ADTN in the absence (open triangles) and presence (filled triangles) of 1.0 μM *cis*-flupenthixol. Salivary glands and adhering ducts and reservoirs from 10 to 12 animals were removed and placed in the salt solution described in the legend to Fig. 1. After removal of the salt solution, the salivary glands were homogenized in 1 ml of a solution containing 2 mM Tris (pH 7.4) and 2 mM EGTA. Adenylate cyclase activity was determined in a previously described (Cote *et al.*, 1981) assay system containing (in a volume of 60 μl) 80 mM Tris HCl (pH 7.4), 10 mM theophylline, 1 mM $MgSO_4$, 0.8 mM EGTA, 0.25 mM ATP, 0.01 mM GTP, salivary gland tissue (30 to 35 μg protein), and drugs as indicated. The assay of enzyme activity was initiated by adding the homogenized tissue (in a volume of 10 μl) to the otherwise complete assay system. The assay system was incubated for 7 min at 30°C; the assay was terminated by boiling the assay system for 2 min. The amount of cAMP was determined by the method of Brown *et al.* (1971). Protein was determined by the method of Lowry *et al.* (1951). The affinity (K_i) of *cis*-flupenthixol for the receptor regulating adenylate cyclase was calculated from the relationship (Cheng and Prusoff, 1973) $K_i = i[(K_m'/K_m) - 1]^{-1}$, in which K_m and K_m' are the concentrations of agonist causing half-maximal stimulation of enzyme activity in the absence and presence of antagonist, respectively, and i is the concentration of antagonist used in the experiment (1 <μM). Data represent means ± SE (N = 4).

of ADTN to enhance adenylate cyclase activity was diminished by *cis*-flupenthixol (Fig. 2, right). In the presence of 1 μM *cis*-flupenthixol, the molar potency of ADTN was diminished approximately 30-fold but the maximal response to the agonist was not attenuated. This suggests a competitive interaction between agonist and antagonist for the receptor regulating adenylate cyclase activity. Assuming a competitive interaction, the affinity of *cis*-flupenthixol for the receptor was calculated to be 90 nM. *Trans*-

Table I. Inhibition of Dopamine-Stimulated Adenylate Cyclase Activity in Homogenates of *N. cinerea* Salivary Gland[a]

Antagonist	Adenylate cyclase activity (pmol cAMP/mg protein/min)	
	No dopamine	10 μM dopamine
None	2.5 ± 0.3	12.7 ± 0.4
Cis-flupenthixol	2.8 ± 0.3	3.3 ± 0.2
Phentolamine	2.8 ± 0.2	8.3 ± 0.2
Propranolol	2.1 ± 0.2	8.1 ± 0.4

[a] Adenylate cyclase activity in homogenates of *N. cinerea* salivary gland was assayed as described in the legend to Fig. 2. Each antagonist was tested at a concentration of 10 μM. Data represent mean ± SE ($N = 4$) of data obtained in a single experiment.

flupenthixol was 30-fold less potent as a dopamine antagonist than its geometric isomer (not shown). The stimulatory effect of dopamine upon adenylate cyclase activity was partially antagonized by phentolamine, an α-adrenergic antagonist, as well as by propranolol, a β-adrenergic antagonist. In the same experiment, *cis*-flupenthixol completely abolished the stimulatory effect of dopamine upon adenylate cyclase activity (Table I).

Either exogenous dopamine or stimulation of the salivary duct nerve (which uses dopamine as its neurotransmitter) hyperpolarizes (House and Smith, 1978; Bowser-Riley and House, 1976; Ginsborg *et al.*, 1976) and elicits fluid secretion from the peripheral cells of the salivary gland (Smith and House, 1977; House and Smith, 1978). ADTN mimicks and cis-flupenthixol blocks the hyperpolarizing response to each of these stimuli (House and Ginsborg, 1976). Although the effects of dopamine, ADTN, *cis*-flupenthixol, and phentolamine upon membrane potential and fluid secretion (House and Ginsborg, 1976) parallel the effects of these compounds upon cAMP production by this tissue, there is no evidence that cAMP acts as a second messenger in fluid secretion (see section V.5.b. of House, 1980). Because the cellular locus of the dopamine-stimulated production of cAMP (e.g., peripheral cell, central cell, or secretory duct cell) is unknown, speculation about the physiological significance of the dopamine-stimulated increase in cAMP production is premature.

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