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A Pharmacological and Endocrinological Study of Female Insemination in *Phormia regina* (Diptera: Calliphoridae)

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Injections of octopamine, dopamine, and the octopaminergic agonists, clonidine and naphazoline, into the thoracic hemocoel enhanced female insemination in sugar-fed (sexually unreceptive) Phormia regina. *Topical applications of the juvenile hormone (JH) analogue, methoprene, also enhanced female insemination in sugar-fed (sexually unreceptive)* P. regina. *Since JH plays a role in receptivity in protein-fed females, it was originally hypothesized that one agonist, clonidine, enhanced female insemination by acting on the corpus allatum (CA) to increase JH biosynthesis. Two or three doses of the antiallatal agent, precocene II, prior to administration of clonidine, did not inhibit clonidine-enhanced female insemination. Removal of the corpus allatum also did not have a significant effect on clonidine-enhanced female insemination. Measurement of juvenile hormone (JH) biosynthesis/release in corpora allata, which were removed at 1, 3, 5, and 7 h postinjection, revealed that clonidine does not affect JH biosynthesis/release. Our study reveals a possible role for the biogenic amines in female insemination in insects. We suggest that the octopaminergic agonist, clonidine, acts downstream from the corpus allatum on the regulatory mechanisms involved in female insemination.*

KEY WORDS: clonidine; octopamine; amines; insemination; juvenile hormone; *Phormia regina*.

INTRODUCTION

The biogenic amines, serotonin (5-HT), dopamine (DA), and octopamine (OA), function as neurotransmitters, neuromodulators, and/or neurohormones in insects

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(Evans, 1980). Processes related to mating in insects have been shown to be either modified or stimulated by these amines. Octopamine enhances pheromone production in female *Heliothis* moths (Christensen *et al.*, 1991). In males, OA is involved in the antennal hair erection in *Anopheles stevensi* (Nijhout, 1977) and enhances the sensitivity of Lepidoptera to pheromones (Linn and Roelofs, 1986; Linn *et al.*, 1992; Roelofs, 1995). OA has also been shown to decrease the mating interval (the time period between spermatophore protrusion and courtship stridulation) in the cricket, *Gryllus bimaculatus*, while 5-HT has the opposite effect (Nagao *et al.*, 1991). The effects of OA, 5-HT, and dopamine on female insemination in insects have not been studied.

In the female blow fly, *Phormia regina*, the ingestion of a protein meal is necessary for initiating the pathways that lead to significant increases in juvenile hormone (JH) titer (Zou *et al.*, 1989). Removal of the corpus allatum (CA), the site of JH production, inhibits female insemination (Qin, 1996), thus providing key evidence that the CA plays a role in female insemination in *P. regina*. JH or the CA has also been shown to have a role in mating in other dipteran insects such as *Calliphora vomitoria* (Trabalon and Campan, 1984), *Musca domestica* (Adams and Hintz, 1969), *Glossina morsitans* (Gillott and Langley, 1981), *Aedes aegypti* (Lea, 1968; Gwadz, 1972), *Drosophila grimshawi* (Ringo and Pratt, 1978), and *Drosophila melanogaster* (Bouletreau-Merle, 1973; Manning, 1966).

In sugar-fed *P. regina*, female insemination is a rare occurrence (Stoffolano, 1974). In the present study, we conducted a pharmacological and endocrinological investigation on female insemination in sugar-fed *P. regina*. Based on the earlier studies, which have shown that biogenic amines can either stimulate or modify mating behavior in insects, we attempted to determine whether or not aminergic agonists are able to enhance female insemination in sugar-fed *P. regina*. Since JH plays a role in female insemination in protein-fed *P. regina*, we investigated the possibility that the application of the JH analogue, methoprene, is able to enhance female insemination in sugar-fed *P. regina*.

EFFECTS OF AMINERGIC AGONISTS ON INSEMINATION

Materials and Methods

Phormia regina was reared and maintained as described previously (Stoffolano, 1974). The flies used in this study all emerged within an 8-h time period. Flies were anesthetized with CO_2, placed on ice, and separated by sex.

Males were placed into a 23 × 23 × 23-cm holding cage and provided with a solution of 4.3% sucrose. The males were provided with fresh beef liver daily from day 1 up through day 4 (day 0 begins at 0 h, day 1 at 24 h, etc.).

On day 5, three males each were placed together into 20 cups (mating containers), each measuring 6.5 cm high and 9.5 cm in diameter. Each mating container had a 1 × 1.5-cm door which was sealed shut by clear, cello tape. The mating container was kept in an upside down position throughout the experiment. A 4 × 4-cm cup of 4.3% sucrose was placed below the 6.5 × 9-cm container and a small hole was made in the center of the cup's cover. A wick was pushed through the hole in the cover. The upside down mating container was balanced on top of the cup of sucrose so that sugar solution was provided to the males housed in the container. Males were kept in these containers up to and through mating. Twenty containers were prepared for each trial.

Females were placed into a 23 × 23 × 23-cm holding cage and provided with 4.3% sucrose up to and through the mating period. Dead females were removed daily and a paper towel used to cover the bottom of the cage was changed daily. These two measures were taken to reduce the chances of the fly making contact with a protein source.

The method of injection involved no physical handling or anesthesia, which tend to affect receptivity (Evans *et al.*, unpublished results). A holding device measuring 10 × 1 × 1.5 cm was made from a tuberculin syringe box and fitted with a piece of Styrofoam, which served as a plunger. One end of the box was fitted with a 7.5 × 7.5-cm piece of nylon cloth. A rubber band was used to tighten the cloth around the box. At 152–154 h postemergence, the fly was collected by holding the box in the experimenter's left hand. The end of the box fitted with the cloth faced to the left and the open end of the box faced to the right. An incandescent lamp was placed approximately 15 cm away from the end of the box fitted with the cloth. A 6.5 × 9.5-cm plastic cup holding the female(s) was placed against the side of the open end of the box. At various times, the 1 × 1.5-cm door to the mating container was opened and one female was permitted to fly toward the light (the end of the box fitted with the cloth). The Styrofoam plunger was then pushed into the open end, trapping the female in the box.

The plunger was pushed to prevent any movement on the part of the fly. Injections took place using a 250- or a 500-μl tuberculin syringe fitted with a 30-gauge needle and driven by a microapplicator (Brookhart *et al.*, 1987). The instrument was calibrated to deliver 1 μl of 145 m*M* NaCl using a 2-μl capillary pipette. Drugs were dissolved in 1 μl of 145 m*M* NaCl unless specified otherwise. All drugs tested were obtained from Sigma Chemical Co. (St. Louis, MO). Controls received 1 μl of 145 m*M* NaCl. Between 2 to 15 min following injection, the female was permitted to fly into the mating container which contained the three males.

After the female and the three males were placed together, the mating container was placed into a photochamber at 28°C with constant light. Constant

light was used to avoid the normal dark period, which occurred at night, and which exposed the flies to a 15-h light period for mating. The next female was injected and the same procedure followed until all 20 females were injected (10 saline-injected and 10 drug-injected). After 15 h, mating containers were taken out of the chamber and placed into the freezer. The spermathecae were removed the next day, placed on a slide in a drop of *Phormia* saline (Chen and Friedman, 1975), and crushed. A phase-contrast microscope (400×) was used to observe the spermathecae for the presence or absence of sperm. Three replicates were performed for each drug.

Results

Dosages of OA (75 μg) and DA (50 μg) produced insemination percentages (56.0 and 22.2%, respectively) which were both significantly different from the controls (3.3% insemination accompanying both amine tests) (Table I). After injection with either OA or DA, flies seldom walked or displayed any movement for at least 40 min postinjection. Higher doses of these drugs (125 to 200 μg) resulted in a greater (>25%) mortality than was acceptable for this experiment. The percentage of females inseminated after treatment with 50 μg of 5-HT was not significantly different from the controls (10%, as opposed to 3.3%) (Table I). Lower doses (30 μg) of OA, DA, and 5-HT had no effect on female insemination compared to the controls (Table I).

Table I. The Effect of Biogenic Amines and Specific Agonists on Female Insemination in Sugar-Fed *Phormia regina* (152–154 h of Age)[a]

Drug	Dose (μg)	% insemination		% mortality
		Saline-injected	Drug-injected[b]	
Octopamine	30	3.3	3.3	0.0
	75	6.7	56.0**	16.7
Dopamine	30	3.3	6.7	0.0
	50	3.3	22.2*	10.0
Serotonin	30	6.7	6.7	0.0
	50	3.3	12.0	16.7
Clonidine	20	6.7	46.4**	6.7
Naphazoline	15	0.0	24.5**	2.0
Naphazoline + clonidine	5 + 20	3.3	51.9**	10.0

[a] Three replicates of 10 saline-injected and 10 drug-injected females per replicate were performed with the exception of naphazoline, for which five replicates were performed.

[b] Insemination percentages significantly different from the saline-injected females by the chi-square test are indicated by asterisk superscripts (*$P < 0.05$; **$P < 0.001$).

Clonidine (20 μg) and naphazoline (15 μg), respectively, produced insemination percentages that were significantly different from the controls (46.4 versus 6.7% and 24.5 versus 0.0% (Table I). Time-response curves showed that clonidine did not have a statistically significant effect on insemination until some time between 4 and 8 h after injection (Fig. 1). Naphazoline, however, exerted its effects later (i.e., between 8 and 15 h postinjection) (Fig. 2). For 10 min post-injection with clonidine, flies displayed little movement. By 15 min postinjection, flies appeared nearly normal. Naphazoline-treated flies appeared normal within 5 min postinjection.

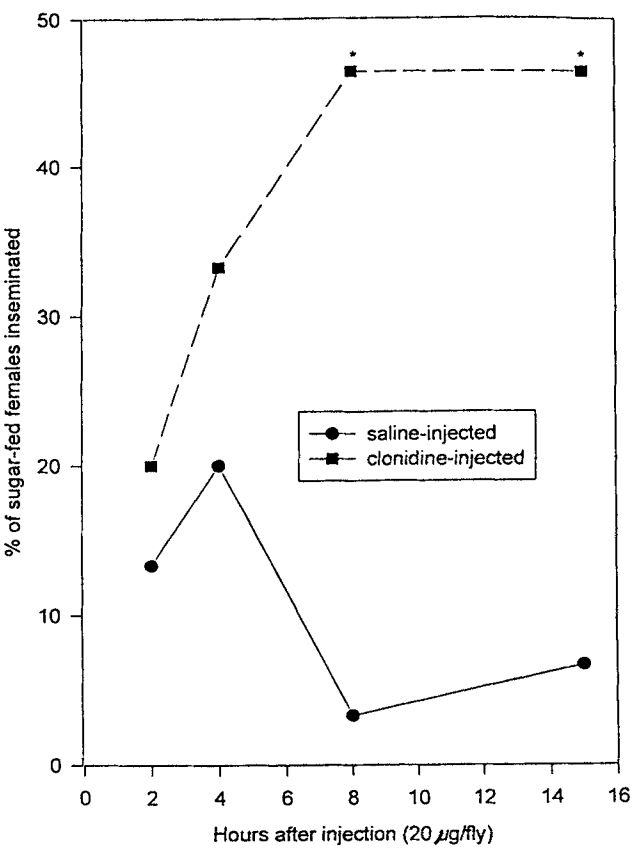

Fig. 1. The mean percentages of sugar-fed, female *Phormia regina* (152–154 h of age) inseminated at various times following injection with clonidine (20 μg) (n = 30 for each point). Squares indicate clonidine-injected females and circles indicate saline-injected females. Insemination percentages significantly different (chi-square tests; $P < 0.001$) from those of the saline-injected females are indicated by an asterisk.

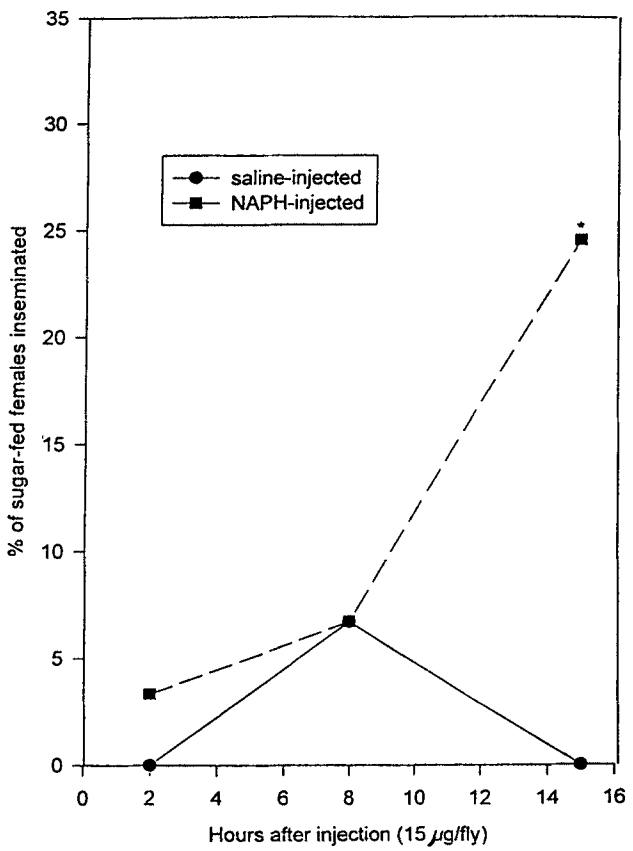

Fig. 2. The mean percentages of sugar-fed, female *Phormia regina* (152–154 h of age) inseminated at various time periods following injection with naphazoline (15 μg) (n = 30 for each point). Squares indicate clonidine-injected females and circles indicate saline-injected females. Insemination percentages significantly different (chi-square tests; $P < 0.001$) from those of the saline-injected females are indicated by an asterisk.

Naphazoline (5 μg) and clonidine (20 μg), administered simultaneously, had little enhancing effect (51.9% insemination in treated females and 3.3% in the saline controls) compared to clonidine by itself (46.4% in treated females and 6.7% in the saline controls) (Table I).

Since clonidine and naphazoline had statistically significant effects on female insemination, the OA antagonists, metoclopramide and yohimbine (Evans, 1981), were administered simultaneously by injection to determine whether these drugs were able to inhibit the insemination-enhancing effects of clonidine and/

Table II. The Effects of Antagonists on the Insemination-Enhancing Effects of the Agonists, Naphazoline and Clonidine, in Sugar-Fed, Female *Phormia regina* (152–154 h of Age)[a]

Dose of agonist + antagonist pair	% insemination		
	Saline	Agonist	Agonist + antagonist
Naphazoline (15 μg) + metoclopramide (20 μg)	0.0 (0.0)[b] A	20.7 (3.3)B	17.2 (3.3)B
Clonidine (20 μg) + yohimbine (10 μg)	13.3 (0.0) A	48.1 (10.0) B	39.1 (23.3) B
Clonidine (20 μg) + metaoclopramide (5 μg)	6.7 (0.0) A	40.0 (0.0)B	50.0 (13.3) B

[a]One replicate consisted of 10 saline-treated, 10 agonist-treated, and 10 agonist + antagonist-treated females. Three replicates were performed.
[b]Percentage mortality is indicated in parentheses.
*Percentages within rows not followed by the same letter indicate significantly different insemination percentages, chi-square tests; $P < 0.02$.

or naphazoline alone. Yohimbine (10 μg) slightly inhibited the insemination-enhancing effects of clonidine (20 μg) (39.1% insemination with clonidine/yohimbine and 48.1% insemination for clonidine alone) when administered simultaneously in 2 μl of 145 m*M* NaCl, but this inhibition was not statistically significant (Table II). Mortality among the clonidine/yohimbine-injected females was quite high (greater than 20%). Metoclopramide (5 μg) did not have a significant effect on clonidine-enhanced insemination (50% insemination with clonidine/metoclopramide and 40% insemination with clonidine alone). A 5-μg dose of metoclopramide was used since a dose of 10 μg in combination with clonidine produced greater than 50% mortality (data not shown). In addition, metoclopramide (20 μg) failed to suppress the insemination-enhancing effects of naphazoline (15 μg) (17.2% insemination with metoclopramide/naphazoline and 20.7% insemination with naphazoline). These results show that aminergic agonists can induce mating in sugar-fed, female *P. regina* which normally do not mate.

EFFECT OF A JH ANALOGUE ON INSEMINATION

Materials and Methods

Since JH plays a role in receptivity in protein-fed females (Qin, 1996), we administered the JH analogue (*s*-methoprene) to sugar-fed females to determine if it would enhance receptivity. Within 4 h after emergence, flies were anesthe-

tized with CO_2, placed on ice, and separated by sex. Both sexes were maintained and reared as previously described. The analogue, *s*-methoprene (90% pure, a gift from Zoecon Corp.), was dissolved in acetone to a concentration of 5 μg/μl. To determine the effect of methoprene on female insemination, three treatment groups were set up as follows.

Acetone (control): 2 μl topically applied at 4 h and again at 75 h postemergence.

Methoprene: 1 μl (5 μg/μl) topically applied at 4 h and again at 75 h postemergence.

Methoprene: 2 μl (5 μg/μl) topically applied at 4 h and again at 75 h postemergence.

Four hours was chosen since this is the most effective time to show precocene's effect on preventing oocyte development (Yin *et al.*, 1989), while 75 h was about halfway through the experiment.

Prior to each methoprene application, flies were anesthetized with CO_2 and placed on ice. Methoprene was topically applied to the ventral thorax. At 152–154 h postemergence, each female was placed with three males for a period of 15 h using the procedure discussed above (except that no injection occurred) and spermathecae examined for sperm. Three replicates were performed.

Results

The JH analogue methoprene enhanced female insemination in sugar-fed flies at doses of either 10 μg (2 × 5 μg) or 20 μg (2 × 10 μg) (82 and 78.9% insemination, respectively), while none of the acetone controls were inseminated (Table III). Less than 3% of the flies administered 20 μg of methoprene died during the mating period. There was no mortality during the mating period among the females treated with 10 μg of methoprene.

Table III. The Effect of Methoprene (JHA) on Female Insemination in Sugar-Fed *Phormia regina* (152–154 h of Age)

Treatment	#/*n*[a]	% insemination*	% mortality[b]
Acetone	0/39	0.0 A	0.0
Methoprene (2 × 5 μg)	32/39	82.0 B	0.0
Methoprene (2 × 10 μg)	30/38	78.9 B	2.6

[a] The number of females inseminated over the total number of females through three trials.
[b] The mortality observed prior to placing the female and males together.
* Percentages within the same column not followed by the same letter indicate significantly different insemination percentages, chi-square tests; $P < 0.001$.

EFFECT OF PRECOCENE ON CLONIDINE-ENHANCED FEMALE INSEMINATION

Materials and Methods

The antiallatal agent, precocene II, inhibits CA development in *P. regina* (Yin *et al.*, 1989). Since the octopaminergic agonist, clonidine, and the JH analogue, methoprene, both enhance female insemination, this experiment utilized precocene to inhibit JH synthesis in order to test the hypothesis that clonidine acts via the CA to enhance female insemination in sugar-fed flies. If this hypothesis is correct, percentages of insemination among the precocene + clonidine-treated females should be significantly less than that of the acetone + clonidine-treated females.

Within 4 h after emergence, flies were anesthetized with CO_2, placed on ice, and separated by sex. At 4 h, 1 μl of precocene II (20 μg/μl; Sigma Chemical Co.) was topically applied to the ventral thorax of each female. Controls received 1 μl of acetone only. Prior to administration, precocene solution was removed from the freezer and warmed to room temperature since the drug forms a precipitate at $-20°C$ (Yin *et al.*, 1989). Flies were maintained and reared in the same manner as discussed previously.

Females were given either two doses (20 μg at 4 and 75 h) or three doses (20 μg at 4, 75, and 128 h) of precocene. Beginning at 152–154 h postemergence, precocene- and acetone-treated females were injected with saline (1 μl of 145 m*M* NaCl) or clonidine (20 μg in 1 μl). Four groups were established: (a) saline and acetone, (b) saline and precocene, (c) clonidine and acetone, and (d) clonidine and precocene.

One female was placed with three males, as described previously, for a period of 15 h beginning at 152–154 h postemergence, and spermathecae were examined for sperm. Three replicates were performed.

Results

More of the clonidine + acetone-treated females in group 1 were inseminated than the saline + acetone-treated females (35.6 versus 2.2%) (Table IV). Fewer of the saline + precocene-treated females in group 1 (two doses of precocene) were inseminated than the clonidine + precocene-treated females (5.1 versus 46.2%). A chi-square test found statistically significant differences among the four treatments ($P < 0.001$). Precocene did not block the insemination-enhancing effects of clonidine since a 2×2 chi-square test found 35.6 and 46.2% not to be significantly different.

When three doses of precocene were administered (Group 2), similar data emerged. The percentages of insemination among the clonidine + acetone- and

Table IV. The Effect of Precocene on Female Insemination Enhanced by Clonidine in Sugar-Fed *Phormia regina* (152–154 h of Age)

Treatment	#/n[a]	% insemination*	% mortality[b]
Group 1 (2× precocene)			
Saline + acetone	1/45	2.2 A,C	2.2
Clonidine + acetone	16/45	35.6 B,D	8.9
Saline + precocene	2/39	5.1 A,C	2.6
Clonidine + precocene	18/39	46.2 B,D	10.3
Group 2 (3× precocene)			
Saline + acetone	0/43	0.0 A,C	0.0
Clonidine + acetone	13/43	30.2 B,D	7.0
Saline + precocene	1/45	2.2 A,C	0.0
Clonidine + precocene	15/45	33.3 B,D	6.7

[a]The number of females inseminated over the total number of females through three trials.
[b]The mortality during the 15-h period the female was with the three males.
*Percentages of each group which are not followed by the same letter are significantly different insemination percentages, chi-square tests; $P < 0.001$.

clonidine + precocene-treated females were significantly different from those of the saline + acetone- and saline + precocene-treated females (30.2 and 33.3 versus 0.0 and 2.2%, respectively) ($P < 0.001$) (Table IV). Once again, precocene did not block the insemination-enhancing effects of clonidine.

EFFECT OF ALLATECTOMY ON CLONIDINE-ENHANCED FEMALE INSEMINATION

Materials and Methods

We have demonstrated that clonidine enhances female insemination in precocene-treated females. We wanted to test this idea further by determining the effect of allatectomy on clonidine-enhanced female insemination, and based on the precocene results, we proposed that the removal of the CA would not affect clonidine-enhanced female insemination.

Flies were reared and maintained as previously described. On day 4 (approximately 96 h postemergence), females were anesthetized with CO_2 and placed on ice for at least 5 min prior to surgery. CA removal was performed according to the procedures of Qin (1996). Allatectomized females (CA^-) were placed into 6.5 × 9.5-cm cups (≤ 7 per cup) containing a kimwipe and a 4 × 4-cm cup of 4.3% sucrose. One day later, the females were released from the cup into a cage. Control (nonallatectomized) females were anesthetized with CO_2, placed on ice, and then placed into 6.5 × 9.5-cm cups. One day later

they were also released from the cups into a cage. The mortality up to clonidine administration at 152–154 h usually averaged about 40% for the CA^- females.

The injection procedure followed that previously outlined. Clonidine hydrochloride (20 μg) was dissolved in 1 μl of 145 m*M* NaCl. Females were injected with either 1 μl of clonidine or saline at 152–154 h. Treatment groups were set up as follows: (a) clonidine and CA^-, (b) clonidine and CA (control); (c) saline and CA^-, and (d) saline and CA (control). Mating trials and spermathecal examination were described previously.

Results

As hypothesized, the percentage of CA/clonidine-treated females inseminated was significantly different ($P < 0.001$) from the percentage of CA/saline-treated females (53.3 and 3.2%, respectively) (Table V). The percentage of CA^-/clonidine-treated females inseminated was significantly different ($P < 0.001$) from the percentage of CA^-/saline-treated females (56.0 and 0.0%, respectively). The two clonidine-treated groups were not significantly different from one another. Mortality among the CA^- flies during the period in which the female was with the three males was 13.3% for the saline-injected females and 16.7% for the clonidine-injected females.

EFFECT OF CLONIDINE ON JH BIOSYNTHESIS/RELEASE

Materials and Methods

To substantiate further the hypothesis that the CA is not involved in clonidine-enhanced, sugar-fed female insemination, JH biosynthesis/release was measured at 1, 3, 5, and 7 h after injection with clonidine. Females were reared and maintained as described previously. Females were anesthetized with CO_2

Table V. The Effect of Clonidine on Female Insemination in Allatectomized, Sugar-Fed Female *Phormia regina* (152–154 h of Age)

Treatment	#/*n*	% insemination*	% mortality[a]
Ca^- + saline	0/27	0.0 A	13.3
CA^- + clonidine	14/25	56.0 B	16.7
CA + saline	1/31	3.2 A	0.0
CA + clonidine	16/30	53.3 B	0.0

[a]The mortality during the 15-h period the female was with the three males.

*Percentages within the same column not followed by the same letter are significantly different insemination percentages, chi-square test; $P < 0.001$.

and placed on ice, and the CC–CA complexes were extirpated at 1, 3, 5, and 7 h after injection with clonidine (20 μg in 1 μl of 145 m*M* NaCl). JH biosynthesis and release were obtained using a previously established radiochemical assay (Liu *et al.*, 1988; Zou *et al.*, 1989). Five CC–CA complexes from each group were tested.

Results

JH synthesis/release steadily increased from 1 to 7 h postinjection in the saline controls, while the synthesis/release remained fairly static during the same time period in the clonidine-treated females (Fig. 3). There were no significant differences in JH biosynthesis/release between the controls and the clonidine-treated females at 1, 3, 5, and 7 h postinjection (Mann–Whitney test).

DISCUSSION

We demonstrated for the first time a possible role for the biogenic amines in female insemination in insects. OA, DA, and the OA agonists, naphazoline and clonidine, enhanced female insemination in sugar-fed, female *P. regina*. Female insemination is a new addition to the list of mating-related processes that are likely to be affected by biogenic amines.

OA and DA only enhanced female insemination at higher doses. Lane and Swales (1978) determined the existence of a blood–brain barrier in the CNS in a closely related species, *Calliphora vicina*. The blood–brain barrier is unlikely to be penetrated by positively charged species (such as the biogenic amines) (Eldefrawi *et al.*, 1968; Hirashima *et al.*, 1994; Milde *et al.*, 1995). Also, the enzyme, *N*-acetyltransferase, is rapidly able to metabolize biogenic amines (Downer and Hiripi, 1994). Hence, if OA is affecting female insemination by acting on the CNS, it seems likely that it would be able to affect this process only at the higher doses that were used here. With such doses, the degree of OA passage across the blood–brain will most likely increase.

The OA antagonists, metoclopramide and yohimbine, failed to block the insemination-enhancing effects of clonidine and/or naphazoline, which might suggest that clonidine and naphazoline do not act on OA receptors. These results are circumstantial since it has been established that yohimbine also has an affinity for 5-HT receptors in insects, such as *Drosophila* (Sadou *et al.*, 1992; Dudai and Zvi, 1984; Roeder, 1994). In addition, metoclopramide acts as an antagonist of DA-induced salivary gland secretion in the cockroach (Evans and Green, 1990). In the future, injecting the antagonist prior to injecting the agonist may produce quite different results since the present results represent the effects of drugs that were administered simultaneously. Taken together, the nonspecific effects of the antagonists and the method of administration of the agonist and

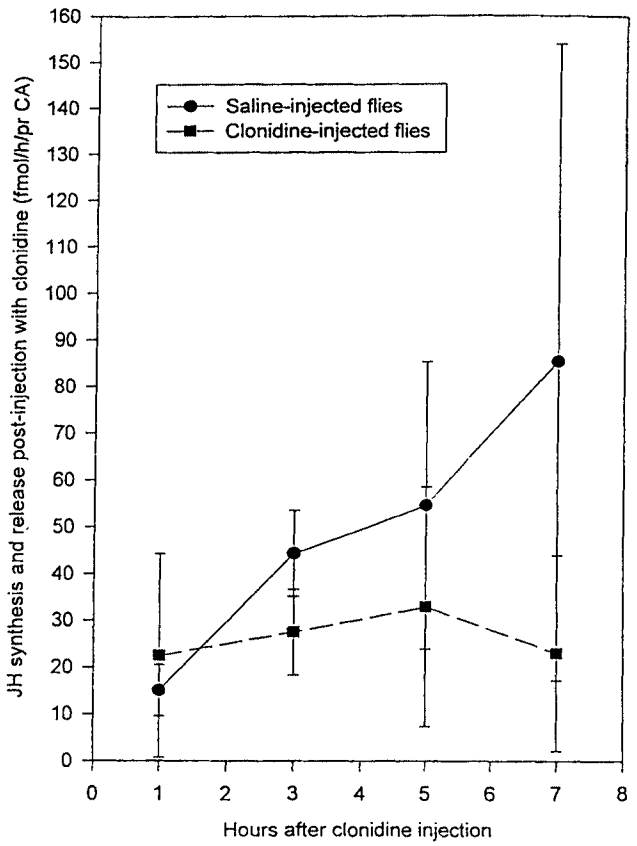

Fig. 3. The effect of clonidine on JH synthesis/release in sugar-fed, female *Phormia regina* (152–154 h of age) at 1, 3, 5, and 7 h postinjection. Squares indicate clonidine-injected females and circles indicate saline-injected females. For each point, three replicates were performed (one replicate consisted of five CC–CA complexes). Means ± SD are indicated. For each point, there are no significant differences between the saline- and the clonidine-injected flies (Mann–Whitney test).

antagonist are two reasons why the agonist/antagonist studies may have failed to reveal the effect, if any, of the antagonist.

The present study demonstrated that methoprene, when administered to sugar-fed females, was able to enhance female insemination. Although JH plays an important role in female insemination in protein-fed flies (Qin, 1996), this was the first time that exogenous administration of JHA has been shown to enhance female insemination in sugar-fed *P. regina*. Methoprene has also been

shown to enhance female insemination in non-protein-fed *Lucilia cuprina* (Barton-Browne *et al.*, 1976).

Since a JH analogue was able to enhance female insemination in sugar-fed females, we initially hypothesized that OA, DA, clonidine, and naphazoline enhanced female insemination by stimulating JH biosynthesis/release from the CA. The antiallatal agent, precocene II, was used to inhibit the development of the CA (Yin *et al.*, 1989). Precocene II inhibits oocyte development (Yin *et al.*, 1989) and female insemination (Evans, unpublished data) in protein-fed *P. regina*, presumably through its ability to inhibit CA development. Results showed that even after the administration of precocene (two or three doses) to sugar-fed females, one of the aminergic agonists, clonidine, still had significant effects on female insemination. Subsequent studies showed that clonidine enhanced insemination in allatectomized females and measurements of JH synthesis/release after injection with clonidine administration revealed that synthesis and release was not significantly affected by clonidine. This is the first study to indicate that an octopaminergic agonist (Evans, 1981) can enhance female insemination in insects and it is able to do so without the CA playing a role.

Since the CA does not seem to perform a role in the insemination-enhancing effects of clonidine, it is proposed that clonidine may be acting downstream from the CA. Clonidine seems to be acting on the regulatory mechanisms involved in female insemination rather than acting to enhance JH synthesis/release.

Based on the results of this study, and previous results from our laboratory, it is possible to speculate on the factors influencing receptivity in *P. regina*. It has already been confirmed that a proteinaceous meal is required for activating the cephalic neuroendocrine system (Yin and Stoffolano, 1990; Yin *et al.*, 1993). A midgut peptide hormone in protein-fed flies is responsible for activating brain neurosecretory cells (Yin *et al.*, 1993). An unidentified neurosecretory factor from the brain will then stimulate the CA to synthesize JH. High titers of JH will activate aminergic mechanisms in the CNS and/or PNS, which will lead to receptive behavior. Presumably, low levels should produce nonreceptive behavior.

The present results demonstrate that the regulatory mechanisms downstream from the CA (the CNS and/or PNS) are already in place regardless of whether or not the female has ingested a protein meal, since clonidine can stimulate sugar-fed females to mate. The nervous system awaits the signal from JH, which can happen only by the ingestion of an adequate protein meal. Further tests need to be conducted to determine what factor is responsible for activating the CA and it must be demonstrated that JH will activate aminergic mechanisms in the CNS and/or PNS.

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