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Species variation in the response of tsetse flies (*Glossina* spp.; Diptera: Glossinidae) to parturition hormone

JAN ŽĎÁREK[1,3] and DAVID L. DENLINGER[2,3]

[1]Institute of Organic Chemistry and Biochemistry, Academy of Sciences of the Czech Republic, Flemingovo nám. 2, 166 10 Praha 6, Czech Republic

[2]Department of Entomology, Ohio State University, 1735 Neil Avenue, Columbus, OH 43210, USA

[3]International Centre of Insect Physiology and Ecology, P.O. Box 30772, Nairobi, Kenya

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Abstract. Parturition hormone, present in the uterus of several species of *Glossina*, causes expulsion of the uterine content in neck-ligated, pregnant females of *Glossina morsitans*, thus eliciting either parturition or abortion. Uteri of all six tsetse species tested (*G. morsitans*, *G. austeni*, *G. brevipalpis*, *G. palpalis*, *G. fuscipes*, *G. pallidipes*) contain parturition hormone activity, and neck-ligated females of *G. morsitans*, *G. austeni*, *G. palpalis* and *G. pallidipes* all respond to the hormone by giving birth. Though uterine extracts of both *G. brevipalpis* and *G. fuscipes* also contain parturition hormone activity, females of these species fail to respond to a hormone injection in our assay system. This suggests that additional or alternative regulatory mechanisms are involved in regulating parturition in certain species.

INTRODUCTION

In tsetse flies, the larva develops within the female's uterus, and at the end of a 9–10 day pregnancy the female gives birth to a single, third-instar larva that pupariates within 1–2 h (Tobe & Langley, 1978; Žďárek & Denlinger, 1993). The fact that brain homogenates or haemolymph from females that have recently given birth elicits a modest parturition response in neck-ligated females (Denlinger et al., 1983; Robert et al., 1984) suggested the presence of a hormonal agent that stimulates parturition. We recently discovered a factor present in the tsetse uterus that is highly active in stimulating parturition when injected into females that are carrying fully grown larvae or abortion when injected into females at earlier stages of pregnancy (Denlinger & Žďárek, 1997). We refer to this active factor as parturition hormone (PH). Interestingly, PH exerts this stimulatory effect only if the female has been neck ligated. This implies that the brain also contributes to the parturition response, but when the neck has been ligated, inhibition from the brain is removed and the female is free to respond to PH. Though *Glossina morsitans* was routinely used as the PH recipient in our earlier study, we demonstrated that uterine extracts from other species of tsetse and even from the common oviduct of the flesh fly, *Sarcophaga bullata* (Sarcophagidae), were also highly active in the *G. morsitans* assay (Denlinger & Žďárek, 1997). In this paper we examine several additional species of tsetse to evaluate their responses to uterine extracts from the same or different donor species.

MATERIAL AND METHODS

Insects

Experiments using *G. morsitans* utilised the colony at International Centre of Insect Physiology and Ecology, Nairobi, Kenya. All other species were from the colonies maintained at the FAO/IAEA laboratory in Seibersdorf, Austria. Certain species, e.g. *G. fuscipes*, were available only in limited numbers. All flies were reared at 25°C with a 12 : 12 light : dark cycle.

Bioassays

Pregnant females containing third instar larvae in utero were neck ligated in the morning (2–3 h after lights on) of the expected day of parturition and injected into the thorax with the test material 5–7 h later. The uterine extracts were prepared by dissecting and homogenising uteri in cold, buffered Locke's saline (Humanson, 1979). The supernatants of centrifuged (10,000 g for 5 min) homogenates were injected in a 1 µl volume. Controls were injected with 1 µl saline. The response to an active extract is usually observed within few minutes after injection. In these experiments we include all parturitions that occurred within 2 h after injection as positive responses.

RESULTS

As previously reported (Denlinger & Žďárek, 1997), we again observed a low incidence of parturition in *G. morsitans* in response to a saline injection, while injection of uterine extracts containing 0.1 uterus equivalents (U.E.) from *G. morsitans* and *G. austeni* elicited a high incidence of parturition (Table 1). In the other five species tested, a saline injection elicited incidences of parturition ranging from 0–22.1%. The relatively high parturition incidences observed with saline controls in *G. austeni* and *G. palpalis* suggest that neither of these species are ideal for use in our bioassay system. Like *G. morsitans*, *G. austeni*, *G. palpalis* and *G. pallidipes* responded to uterine extracts from conspecifics or heterospecifics by giving birth. This, however, was not the case for *G. brevipalpis* or *G. fuscipes*. Neither species responded strongly to uterine extracts from conspecifics, and in the case of *G.*

TABLE 1. Incidence of parturition in different species of tsetse flies in response to injection of uterine extracts from conspecific or heterospecific donors. Females were neck ligated in the morning of the expected day of parturition and injected with either saline or various concentrations of uterine extracts (expressed as uterus equivalents, U.E.). Parturition incidence is reported for the first 2 h after injection.

Recipient	Donor	Amount injected (U.E.)	N	% parturition
G. morsitans	1 µl saline	–	10	10.0
"	*G. morsitans*	0.1	33	60.6
"	*G. austeni*	0.1	36	47.2
G. austeni	1 µl saline	–	77	22.1
"	*G. austeni*	0.1	26	50.0
G. brevipalpis	1 µl saline	–	14	0
"	*G. brevipalpis*	0.2	14	7.1
"	*G. austeni*	0.4	13	0
G. palpalis	1 µl saline	–	24	16.7
"	*G. palpalis*	0.2	20	75.0
G. fuscipes	1 µl saline	–	10	0
"	*G. fuscipes*	0.1	10	10.0
G. pallidipes	1 µl saline	–	18	0
"	*G. pallidipes*	0.1	18	94.5

brevipalpis, the extract from *G. austeni* that was shown to be active in both *G. austeni* and *G. morsitans* also failed to induce parturition. Yet, a uterine extract from *G. brevipalpis* successfully stimulated parturition in *G. palpalis*.

DISCUSSION

These results suggest a wide distribution for PH in the uteri of different tsetse species. And, the fact that the common oviduct of the flesh fly, *Sarcophaga bullata*, also contains PH (Denlinger & Žďárek, 1997) indicates that the distribution of PH goes even beyond the Glossinidae. Yet, it is also clear that PH does not elicit the same response in all species of tsetse. This is especially noticeable in *G. brevipalpis* and *G. fuscipes*, two species that contain PH activity but fail to respond under the conditions of our current assay system. Perhaps additional levels of control prevent the expression of PH activity in such cases or PH may serve some other function. It is already evident in *G. morsitans* that PH does not normally function by itself to stimulate parturition. It can do so only if the female has first been neck ligated. The localisation of activity within the uterus (Denlinger & Žďárek, 1997), however, strongly argues that PH plays some regulatory role within the reproductive system, even in species not showing an overt biological response.

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