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Rapid communication

Drosophila model for in vivo pharmacological analgesia research

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Abstract

Fruit flies (*Drosophila melanogaster*) are typically used for genetic studies but they also could be employed for neuropharmacological research. Therefore, we designed an apparatus and developed methods to investigate how injecting antinociceptive drugs, i.e., a gamma-aminobutyric acid B receptor agonist, to adult flies affects their avoidance of noxious heat stimuli. We found a drug-induced dose-dependent increased threshold for heat avoidance and we propose *Drosophila* as an ethically acceptable animal model for in vivo pharmacological analgesia research.

Keywords: Pain; Nociception; Fruit fly

Only in vivo animal experimentation can lead to successful discovery of new ‘‘pain killers’’. Pain (or nociception) research with animal models is bound by ethical concerns (Le Bars et al., 2001). Fruit flies are increasingly being used in neuropharmacology (Manev et al., 2003). Since no serious ethical controversies have been raised regarding in vivo experiments in insects, it appears that a *Drosophila* model for screening putative analgesics would be advantageous in the discovery of new drugs. To our knowledge, adult fruit flies have not as yet been used for pharmacological pain research.

Discussing the issue of pain in animals inevitably leads to anthropomorphic references. From a practical point of view, the response of an animal to noxious stimuli, for example, heat, and the capacity of a drug treatment to attenuate this response are the usual components of pain research. Thus, mice and rats are exposed to a hot-plate test or their tails are immersed into hot water and the latency to jump or tail-flick, respectively, is measured. Administration of drugs to these animals, including agonists for $GABA_B$ subtype (GABA = γ-aminobutyric acid) neurotransmitter receptors, can prolong latency to heat response (Thomas et al., 1996). *Drosophila* also express $GABA_B$ receptors, which can be activated by the agonist 3-aminopropyl-(methyl)phosphinic acid [3-APMPA] (Dzitoyeva et al., 2003; Mezler et al., 2002). As a proof of principle for using *Drosophila* in pharmacological pain studies, we tested whether injecting flies with 3-APMPA would alter their heat response.

We designed a test apparatus which consists of a spiral plastic tube that forms a tunnel for flies to negotiate—hot water (e.g., 40–60 °C) is pumped through this tube to produce a heat barrier (Fig. 1A). Flies are placed inside an empty plastic ‘‘start’’ tube, which is inserted into the tube holder; the ‘‘end’’ tube with the heat barrier is inserted into the opposite side. The apparatus is placed horizontally—the tip of the ‘‘end’’ tube facing a source of light, (flies prefer light-phototaxis). The apparatus is shaken so that all flies fall into the tip of the ‘‘start’’ tube, and flies are allowed to move for 2 min. The number of flies in the ‘‘end’’ tube and outside the barrier is counted. Statistical analysis was performed by the Fisher’s exact test.

Forty flies (5–7-day-old female Canton S) per group were injected with either vehicle (control) or 3-APMPA as described elsewhere (Dzitoyeva et al., 2003). No vehicle-injected flies passed through the tunnel at 60 °C, whereas 3-APMPA dose-dependently allows the heat barrier to be crossed. At 42 °C, 27.5% of control flies pass through; 2 pmol/fly of 3-APMPA, a dose ineffective at 60 °C, allowed 57.5% of the flies to pass the 42 °C barrier (Fig. 1B). We also tested flies under conditions in which phototaxis was exchanged for geotaxis; i.e., the apparatus was placed vertically

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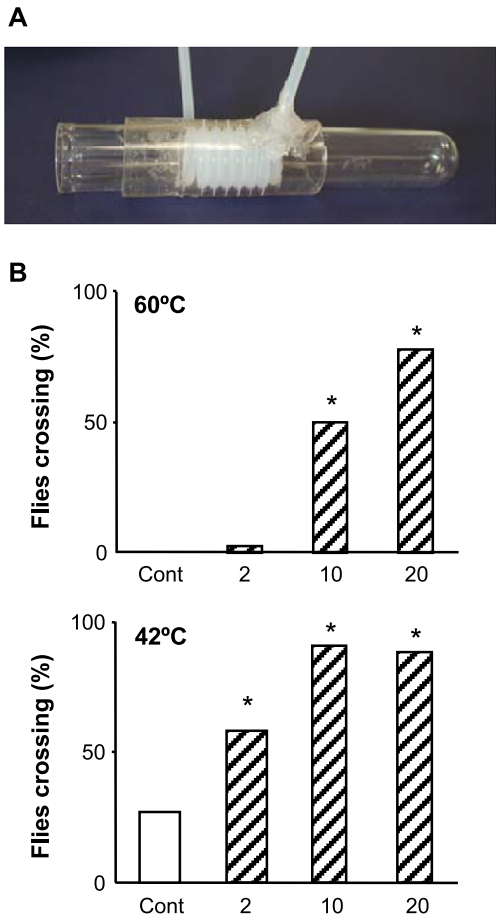

Fig. 1. Intra-abdominal injection into adult *Drosophila* of the $GABA_B$ receptor agonist 3-APMPA reduces heat avoidance behavior. (A) Water (24–60 °C) is pumped through a spiral plastic tube that forms a tunnel for flies to negotiate. (B) Starting 30 min after injection, the flies are tested at the following temperatures (°C): 24, 60, and 42. The interval between the tests is 15 min, and 5 min before each test, flies were kept in the dark. The percentages of flies crossing the heat barrier at 60 and 42 °C are shown. Open bars: control (Cont), striped bars: 3-APMPA (the doses, pmol/fly/0.2 μl, are indicated). $*P < 0.05$ compared with the corresponding control (Fisher's exact test). No significant difference between the groups was observed at 24 °C.

in the absence of light stimulus (flies prefer to climb), and we obtained similar results as in the phototaxis assay (not shown).

Thus, our results suggest that adult *Drosophila* can be used in neuropharmacological nociception research. Our findings in flies are consistent with previous results obtained in rats; i.e., a subcutaneous injection of a $GABA_B$ receptor agonist to rats produced dose-dependent antinociception in both the tail-flick and hot-plate test (Thomas et al., 1996). With the *Drosophila* model we describe here, it will be possible to further characterize mechanisms involved in the action of $GABA_B$ receptor system in heat avoidance.

Although anthropomorphizing the behavioral responses of flies we observed in our present study as *nociception* may be controversial, these fly behaviors are remarkably similar to responses of mammals to heat and to the $GABA_B$ receptor agonists. In fact, the perception of and behavioral responses to low and high temperatures are well developed in *Drosophila* (Sayeed and Benzer, 1996). Recent research in *Drosophila* larvae has identified specific thermosensory neurons (Liu et al., 2003) and also a gene, *painless* (Tracey et al., 2003), encoding a putative heat-sensing receptor/channel. We propose that combining the advantages of gene manipulation in *Drosophila* with the neuropharmacological techniques described here may advance the discovery of new analgesic drugs. Moreover, at this time, it appears that the *Drosophila* model is ethically more acceptable than the in vivo mammalian animal models.

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