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The use of the anaesthetic, enflurane, for determination of metabolic rates and respiratory parameters in insects, using the ant, *Camponotus maculatus* (Fabricius) as the model

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Abstract

This study investigated the effects of the anaesthetic, enflurane, on metabolic rates and ventilation patterns in the spotted sugar ant, *Camponotus maculatus*, using flow-through respirometry. The standard metabolic rate was not affected by the anaesthetic. While the ants were anaesthetised they exhibited a similar discontinuous gas exchange cycle to that observed when they were voluntarily motionless, but their spiracles remained open for a longer time during the open or burst phase even though the amount of CO_2 emitted during this phase remained constant. We discuss this finding in the context of the central nervous system control of the spiracle muscle. For both the determination of standard metabolic rate and ventilation patterns the individual ant has to be motionless. From this study we recommend the use of enflurane to ensure immobility in ants, and other small active insects, during the determination of standard metabolic rates, but the anaesthetic cannot be used to quantify the respiration pattern.

Keywords: Respiration; Metabolic rate; Ant; Discontinuous gas exchange cycle; Anaesthetic

1. Introduction

The standard metabolic rate has been used as a baseline measurement against which comparisons are made, between species and within species regarding different physiological conditions (Eckert et al., 1988), and is defined as the value measured at a particular temperature when an animal is quiet, inactive, not digesting a meal, and not experiencing any stress (Withers, 1992). Ants are important components of several ecosystems and have colonised almost all habitats (Holldobler and Wilson, 1990), thus their standard metabolic rate has been of interest to explain their role in these habitats and their ability to survive under different environmental stresses.

The interspecific allometry of standard metabolic rates in ants was attempted by Lighton and Wehner (1993), they found that excluding the results obtained using apparatus where one was unsure whether the ant was active, e.g. Warburg respirometers gave a better prediction of standard metabolic rate. Nielsen (1986) also discussed the problems of comparing respiratory results from different investigations due to the different methods used. Thus, we consider that temperature and activity are the most important criteria in the determination of standard metabolic rate in ants. Most authors give the temperature at which the metabolic rate was measured and standard metabolic rate can be corrected to a common temperature using Q_{10} (Lighton and Wehner, 1993). For most ant species a reasonable assumption of Q_{10} is 2.0 (Vogt and Appel, 1999). However, activity is more of a problem as unless the individual ant can be viewed, one cannot be certain about the levels of activity. One method of solving this problems is to use an anaesthetic. The anaesthetic, enflurane, has been used by Holm-Jensen et al. (1980) and Nielsen et al. (1982) to determine metabolic rates. They chose this anaesthetic because it did not produce muscular excitation or liberate formic acid. Enflurane is a halogenated methyl-ethyl ether, known to act on the central nervous system, poss-

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ibly via the γ-aminobutryric acid (GABA) receptors (Jones and Harrison, 1993).

In this study we tested whether enflurane is appropriate to use for determination of standard metabolic rates in ants by comparing the values obtained for an inactive adult ant with those obtained for the same ant but anaesthetised. Thus all the other variables remained constant. Recent studies have shown that several adult ants (Lighton 1988, 1990; Lighton and Wehner, 1993) exhibit a distinctive pattern of external gas exchange referred to as "discontinuous gas exchange cycle". This pattern of ventilation had early been found in several other insect species (Punt, 1950; Miller, 1981). This ventilation pattern is disrupted by movement. Thus to quantify and compare the parameters of the discontinuous gas exchange cycle the insect has to be immobile. Surprisingly not all adult insects exhibit this form of respiration and if an insect is completely inactive we would be able to be certain that discontinuous gas exchange cycle was or was not taking place. The second part of this study was to determine whether we could use enflurane for determining whether an ant species does exhibit discontinuous gas exchange cycle and to quantify the parameters of this cycle.

The discontinuous gas exchange cycle is a cyclic discontinuity in external gas exchange previously referred to as discontinuous ventilation cycles. This discontinuous gas exchange typically consists of three phases (Miller, 1981; Kestler, 1985; Sláma, 1988). These are; a closed phase where the spiracles are shut, which inhibits respiratory water loss and negligible external gas exchange takes place. This is followed by the flutter phase, during which slight opening of the spiracles on an intermittent basis allows some O_2 to enter the tracheoles, but little CO_2 or water vapour is lost. Finally, in the open or burst phase, accumulation of CO_2 from respiring tissues triggers some or all of the spiracles to open widely, resulting in the rapid release of CO_2 and water vapour to the atmosphere. Several authors have postulated that the spiracle muscle movements are under central nervous system control (Kaars, 1981; Miller, 1981; Sláma, 1988). Thus enflurane could have an effect on the discontinuous gas exchange cycle.

For this study we chose the spotted sugar ant, *Camponotus maculatus* (Fabricius), which exhibits discontinuous gas exchange to test the effects of enflurane on standard metabolic rates and on the discontinuous gas exchange cycle. Our main aim was to find a means of immobilising ants so that the standard metabolic rate could be accurately measured and the parameters of the discontinuous gas exchange cycle determined.

2. Materials and methods

2.1. Ants

Camponotus maculatus (Fabricius) workers came from a colony collected from Klipriviersberg Nature Reserve, near Johannesburg, South Africa. The colony was housed in a modified Lubbock nest in an insectary at 27°C and supplied with water and sugar solutions ad libitum. The colony was occasionally given dead meal worms. Colonies of this ant species have survived for several years under these conditions in our insectaries.

2.2. Respirometry

Measurements of CO_2 emission were made at 25°C using a flow-through respirometry system in conjunction with a computerised data acquisition package (Datacan V, Sable Systems), described by Lighton (1991). Briefly, in the basic setup ants were placed individually in 25 ml glass respirometry chambers with air flow rates of 100 ml min^{-1} regulated by a mass flow controller (Sierra Instruments). The incurrent ambient air was scrubbed of H_2O and CO_2 by being drawn though drierite and ascarite columns, respectively. This dry CO_2 free stream of air was then drawn through the respirometry chamber and finally through a CO_2 analyser (LiCor LI6262). This setup can be used to accurately determine very small differences in CO_2 content in the airstream. A validation of the accuracy of this system is described by Lighton and Duncan (1995). The ants were initially weighted to ±0.01 mg (Shimadzu Libror AEG-45SM) and the amount of CO_2 produced was taken every 2 s for 30 min. The ants were observed to ensure that they remained stationary during sampling.

Paired runs were performed on 14 worker ants in normal quiescent and anaesthetised states. The procedure was as follows; a baseline recording was made and then the ant was placed in the respirometry chamber and its CO_2 emission was recorded once the ant was stationary, after a 30 min recording the ant was removed and another baseline measurement made. A further baseline measurement was made by drawing the mixture of 5% enflurane in dry air through the system, the ant was then replaced into the respirometry chamber and, once anaesthetised, a 15 min recording was made. The ant was observed to ensure that it remained immobile during the entire recording. On removal from the system the ants recovered within 2 min. As enflurane was found to interfere with the infrared detection of the CO_2 analyser, it was removed using a drierite scrubber placed between the ant and the analyser.

Standard metabolic rates were calculated as the mean CO_2 production rates ($\dot{V}CO_2$) over four to ten complete discontinuous gas exchange cycles (Fig. 1, one cycle is defined as the end of one burst of CO_2, open phase,

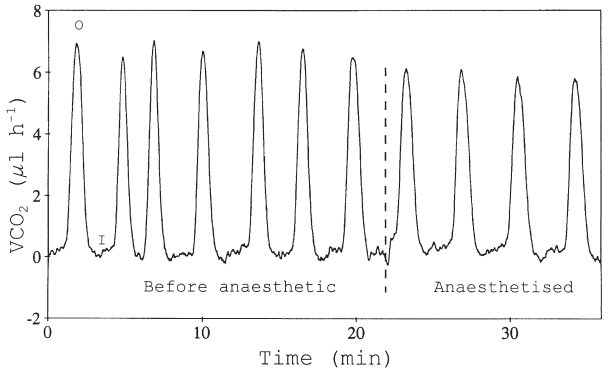

Fig. 1. Recording of CO_2 emission from a 7.40 mg *Camponotus maculatus* ant at 25°C while motionless (before administration of enflurane) and while anaesthetised with enflurane. O marks the open or burst phase and I marks the interburst phase of the discontinuous gas exchange cycle.

marked O, to the end of the following open phase). To express metabolic rate in terms of energy (μW or W kg^{-1}), the respiratory quotient (RQ) was assumed to be 0.72 which had been previously determined for other ant species within the *Camponotus* genus (Lighton and Wehner, 1993). An RQ=0.72 gives an energy equivalent of 26.4 J ml^{-1} CO_2 (Withers, 1992).

The ventilation cycle characteristics (as given in Table 1) were calculated as follows. The rate of burst emission is the volume of CO_2 emitted during the open phase, obtained by calculating the area under the CO_2 peak (marked as O in Fig. 1), and then expressed as a mass specific value, and as a mass and time specific value. The actual volume of CO_2 emitted in each peak, area under the CO_2 peak, is the open phase volume. The burst length is the time taken for CO_2 to be emitted. The frequency of the discontinuous gas exchange cycle is calculated by determining the number of peaks of CO_2 per second, and the period is the duration of one discontinuous gas exchange cycle (i.e. an open and interburst phase, marked as O and I respectively on Fig. 1).

Table 1
Standard metabolic rate and characteristics of the discontinuous gas exchange cycle (mean±SD) in *Camponotus maculatus* ants before and while being anaesthetised[a]

	Before anaesthic	During anaesthetic
Mass (mg)	10.10±3.75	10.10±3.75
N	14	14
Standard metabolic rate:		
μW	12.49±6.11	14.34±8.64
W kg^{-1}	1.52±1.01	1.26±0.53
Rate of CO_2 emission:		
CO_2 (ml h^{-1})	0.00196±0.0012	0.0017±0.0008
CO_2 (ml g^{-1} h^{-1})	0.207±0.14	0.172±0.073
Rate of burst CO_2 emission:		
Burst CO_2 (ml g^{-1})	0.00651±0.0027	0.00823±0.0036
Burst CO_2 (ml g^{-1} h^{-1})	0.3143±0.188	0.2934±0.0973
Burst length (s)	91.54±18.53	98.43±20.01*
Open phase volume (μl)	0.083±0.065	0.071±0.036
Discontinuous gas exchange cycle:		
Frequency (mHz)	7.24±2.26	6.93±1.99
Period (min)	2.54±0.93	2.65±0.99

[a] The sample sizes are the number of ants measured. Significantly different means between columns (using the paired two-sample Student's *t*-test, $p<0.05$) are denoted by an asterisk.

2.3. Statistical methods

Data are presented as means±standard deviation. Sample size (*N*) is indicated in the text as either representing individual ants or in the case of gas exchange characteristics, four to ten discontinuous gas exchange cycles per ant. Statistical analyses were performed using Graphpad Instat ver. 3.00 (1997). The paired Student's *t*-test was used to compare the ventilation characteristics between ants before and while under anaesthetic.

3. Results

3.1. Standard metabolic rate

Results are summarised in Table 1. Comparison of the standard metabolic rates of ants while inactive compared to "forced" inactivity (anaesthetised) indicated that neither the metabolic rate (μW: $t=0.83$, $p=0.42$) nor the mass specific metabolic rate (W kg^{-1}: $t=0.99$, $p=0.34$) was altered by the anaesthetic (Table 1). The values obtained for standard metabolic rate while the ant was inactive compared to that obtained while the ant was under anaesthetic did not differ significantly, and it is highly unlikely that there would be a difference in the standard metabolic rate if the sample size was increased.

The standard metabolic values we obtained were also not significantly different to those predicted using the general equation relating ant standard metabolic rate at rest to mass at 25°C proposed by Lighton and Wehner (1993). The equation is: $SMR=1143\ M^{0.933}$, where SMR is standard metabolic rate in μW and *M* is live body mass in g. This equation predicted a value of 15.7 μW for the standard metabolic rate for these ants. However, the standard metabolic rate predicted by the equations of Vogt and Appel (1999) were significantly higher than that measured.

3.2. Discontinuous gas exchange cycle

A typical discontinuous gas exchange cycle for a *C. maculatus* worker is shown in Fig. 1. In these ants it was difficult to separate the flutter and closed phases, thus in the analysis they were grouped together and are referred to as the interburst phase (marked I in Fig. 1).

As this ant species lives in a mesic environment it is not unexpected that there is no long and well-defined closed phase as is found in ant species living in arid regions.

The ventilation cycles obtained from a stationary ant before the anaesthetic was applied look similar to those obtained while the ant was being anaesthetised (Fig. 1). Analysis of the various parameters indicated that the only significant difference in these cycles was the duration that the spiracles were open during the open phase (t=5.44, $p<0.05$, Table 1). The burst length of the open phase was significantly longer while the ant was anaesthetised. All other discontinuous gas exchange cycle parameters were unaffected by that state of the ant. The mean volume of CO_2 emitted during the open phase remained similar even though the time that the spiracles were open differed. This suggests that the time the spiracles remained open was not important for ensuring that all the CO_2 was expelled.

The duration of the two phases as a percentage of the total discontinuous gas exchange cycle duration were calculated by regression analysis, which is the linear regression between duration of discontinuous gas exchange cycle and phase length. The open phase occupied 29.7% (±4.3% SE) of total discontinuous gas exchange cycle duration before the ants were anaesthetised, and 28.2% (±5.3% SE) while the ants were under the anaesthetic. Using ANCOVA there was no significant difference. The duration of the interburst phases as a percentage of the discontinuous gas exchange cycle also were not significantly different in the ant before anaesthetic and while anaesthetised (60.2%±6.1% SE and 76.6%±10.5% SE, respectively).

4. Discussion

4.1. Standard metabolic rate

Our results indicated that the anaesthetic, enflurane, had no effect on the determination of standard metabolic rate in *Camponotus maculatus*. Thus, if our results can be extrapolated to other ant species, immobilising ants using enflurane could eliminate some of the variability found in the standard metabolic rates measured to date. It is feasible that enflurane could also be used for respirometry studies of other small active arthropods.

We compared the standard metabolic rate value we obtained for our mesic Camponotus ant species with those reported for other Camponotus species (Table 2). All the values in this table were obtained using flow-through respirometry except for *C. herculeanus* where a Warburg respirometer was used. Thus these values could give an indication whether ants belonging to this genus lower their metabolic rate in response to arid environments. The standard metabolic rate obtained for the ant in this study is similar to that obtained for the other mesic species, *C. vicinus*, but the values are lower than those obtained for the two arid adapted species. Only the tropical ant, *C. sericeiventris*, conforms to the proposed theory linking metabolic rates and environment. This implies that further results are needed on ant metabolic rates before we can clarify the relationship between metabolic rate and environment in ants, and these measurements need to be taken under controlled temperature using immobile ants.

Table 2
Comparison of the standard metabolic rate obtained for *Camponotus* ant species from different habitats

Species	Habitat	Live mass (mg)	Standard metabolic rate[a] (W kg^{-1})
C. herculeanus[1b]	Subarctic	41.2	1.89
C. fulvopilosus[2]	Arid	43.0	1.40
C. detritus[3]	Arid	44.4	1.46
C. vicinus[4]	Mesic	50.0	1.19
C. maculatus[5]	Mesic	10.1	1.22
C. sericeiventris[6]	Tropical	40.2	2.29

[a] Standard metabolic rate was corrected to 25°C assuming a Q_{10} of 2.0.

[b] Sources are [1]Nielsen et al. (1982); [2]Lighton (1989); [3]Lighton (1990); [4]Lighton (1988); [5]this study; [6]Lighton and Gillespie (1989).

4.2. Discontinuous gas exchange cycle

Activity disrupts the discontinuous gas exchange cycle (Lighton, 1996) thus an inactive insect is required to accurately quantify parameters of this ventilation. To completely immobilise their study ant Lighton et al. (1993) decapitated the ant and sealed the wound with low-melting-point wax. These decapitated ants started to ventilate discontinuously within a few minutes and their ventilation characteristics were almost identical to those of motionless intact ants. Thus decapitation is a method which could be used for ensuring immobility while measuring the breathing patterns of ants. The disadvantage of this method is that the ant cannot be manipulated (e.g. placed in different environments) and then tested for changes in its discontinuous ventilation cycle. Anaesthetising ants does not have this disadvantage as the periods of immobility will be determined by the researcher. However, from this study we found that anaesthetics may interfere with the nervous control of the discontinuous gas exchange cycle, see discussion below. The only present method to ensure inactivity during measurements is to monitor the activity of the study animal.

4.3. Control of discontinuous gas exchange

The results from this study will contribute to the understanding of the mechanisms of the ventilation con-

trol in ants, which is still poorly understood (Lighton, 1996). The time that the spiracles were fully open was significantly longer while the ant was given the anaesthetic, forced inactivity, than while it was voluntarily inactive. From his studies on discontinuous gas exchange in grasshoppers, Harrison (1997) concluded that elevation of tracheal CO_2 levels to a threshold appears to trigger the spiracular open phase. This agrees with the studies on several ant species, by Lighton (1996) in which he found that CO_2 accumulation is the primary determinant of open phase initiation and the ventilation cycle frequency. Thus it is postulated that the spiracle muscle motoneurones are firing in response to levels of hypoxia. The concentration of CO_2 which can be tolerated by the insect in its tracheoles depends on the haemolymph buffering capabilities of the insect. In the experiment using the anaesthetic the same ant was used so there would be no change in the levels of CO_2 which cause the spiracles to open, also the same amount of CO_2 would be expelled. This was verified by our results (Table 1). This would also explain why the discontinuous gas exchange cycle frequency remained the same. What we need to ask is what causes the spiracles to close. In the anaesthetised ants the spiracles remained open for longer than was necessary to expel the necessary volume of CO_2, thus, we suggest that the mechanism causing the spiracles to close was affected by the anaesthetic.

From their study in mammal brain tissue, Jones and Harrison (1993) concluded that enflurane prolonged the time course of GABA receptor-mediated synaptic inhibition. Prolongation of synaptic inhibition in the central nervous system is consistent with the physiological effects that accompany anaesthesia. GABA is the principle inhibitory neurotransmitter both in the central nervous system and at nerve/muscle junctions in insects (Chapman, 1998). As there are many similarities between invertebrate and vertebrate (for review see Sandman, 1999), it would be reasonable to assume that enflurane may be acting in the same or a similar way in insects. Although enflurane probably prolongs synaptic inhibition in the central nervous system, the spiracles will still close as the ventilation cycle can be generated in the absence of the central nervous system (Miller, 1981). The time taken for the spiracles to close would be longer. This is a possible explanation for the longer time the spiracles spent fully open during the discontinuous gas exchange cycles in anaesthetised ants. If most anaesthetics act by prolonging the synaptic inhibition in the central nervous system then it will not be possible to use anaesthetics for studying the discontinuous gas exchange cycles in insects.

In conclusion, if we can extrapolate our study to other ant species, or even other insects, we recommend the use of enflurane to ensure immobility in insects during the determination of standard metabolic rates especially when the insect cannot be monitored or movement detected. Thus this method will be useful for determining metabolic rates for ecological studies. However, this anaesthetic does have some affect on the respiratory gas exchange pattern found in several insects, so cannot be used in the quantification of ventilation patterns. Therefore, in studies to determine whether there is a link between ventilation patterns and environment this method will not be appropriate.

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