THE ROYAL SOCIETY

Effects of morphine on electrically evoked contractions of the vas deferens in two congeneric rodent species differing in sperm competition intensity

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An early prediction of sperm competition theory was that males should adjust the number of sperm they deliver according to the risk of double mating and this has received empirical support in recent years. It has been suggested that adaptive regulation of sperm delivery in mammals may depend on changes in vas deferens contractility. In laboratory mice, the vas deferens is sensitive to opioid agonists and the secretion of endogenous opioid peptides can be affected by social interactions that may be predictive of sperm competition risk. The present experiment was conducted to determine whether morphine, an opioid agonist (at the μ-receptor), has different effects on electrically evoked contractions of the isolated vas deferens in two congeneric rodent species differing in sperm competition intensity. Morphine inhibited contractions of the vas deferens in the non-monogamous deer mouse (*Peromyscus maniculatus*) but not the monogamous California mouse (*Peromyscus californicus*). This implies that the vas deferens of *P. maniculatus* possesses functional μ-receptors and, thus, should be able to respond to changes in the circulating levels of endogenous agonists whose secretion can be affected by social interactions predictive of sperm competition risk.

Keywords: morphine; sperm competition; vas deferens; *Peromyscus*; opioids; electrical stimulation

1. INTRODUCTION

Sperm competition is the competition between the sperm of different males to fertilize a single female's gamete(s) (Parker 1970). In a species with internal fertilization, sperm competition may occur whenever a female engages in 'double mating' such that live sperm from two (or more) males are present within her reproductive tract. Under these circumstances, notwithstanding mating order effects, a 'raffle principle' applies, i.e. a given male can increase the probability of siring her offspring by inseminating more sperm (Parker 1970, 1990*a*). However, for males there is a trade-off between the non-trivial cost of ejaculate production (Dewsbury 1982) and the risk of sperm competition. Following from this, an early prediction of sperm competition theory was that males should inseminate more sperm when the risk of double mating and, hence, of sperm competition is high (Parker 1982, 1990*a*,*b*; Baker & Bellis 1995). This prediction has since been confirmed for a number of insect and mammalian species (for a review see Parker *et al*. 1997). For example, it has been reported that male laboratory rats (*Rattus norvegicus*) ejaculate more sperm when mating with a female that they have not previously guarded (Bellis *et al*. 1990).

It has been suggested that adaptive regulation of ejaculate composition might depend on changes in the contractility of the vas deferens (Baker & Bellis 1995). Contractions of the distal portion of this duct are critical for sperm delivery (Batra 1974; Guha *et al*. 1975; Hib *et al*. 1982). Therefore, the number of sperm ejaculated might depend on the proportion of the sperm stored in the vas deferens that are loaded into the urethra during emission just before ejaculation. If this is the case, species differences in the ability of the vas deferens to respond to changes in the social environment should be expected. In species in which sperm competition has been a chronic selective force, the contractility of the vas deferens should be systematically related to the risk of sperm competition as indicated by changes in the social environment.

While species differences in vas deferens physiology have not been widely studied, there is evidence of genotype-dependent responses to opioid agonists in the vas deferens of inbred strains of laboratory mice (*Mus musculus*). Morphine, a μ-receptor agonist, inhibits contractions of the isolated mouse vas deferens induced by electrical stimulation of the intramural nerves (Henderson *et al*. 1972). However, its effects are more potent in the DBA/2 strain than in the C57BL/6J strain (Berti *et al*. 1978). Such differences are difficult to explain in evolutionary terms since they are primarily the result of artificial selection within laboratory breeding programmes. Nevertheless, genotype-dependent responses to opioid agonists are particularly interesting since endogenous opioid peptides (EOPs) are involved in the regulation of male reproductive function at multiple sites within the hypothalamic–pituitary–testicular (HPT) axis (Fabbri *et al*. 1989). Furthermore, since EOP secretion can be modulated by social stressors, these peptides may be involved in the adaptive modulation of various aspects

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of male reproductive physiology in response to changes in the social environment (Knol 1991).

In laboratory mice, social conflict within resident–intruder dyads produces potent opioid-dependent analgesia in subordinates (Rodgers & Hendrie 1983), which is naloxone reversible and cross-tolerant with morphine (Rodgers & Randall 1985). Moreover, dominants exhibit opioid-dependent reversible hyperalgesia (Rodgers & Hendrie 1983), a finding that has been replicated in laboratory rats (Raab *et al.* 1986). Pituitary secretion of EOPs into the peripheral circulation has been implicated in the antinociceptive responses to social conflict seen in defeated rodents. In particular, fighting (Huhman *et al.* 1990) and submission (Huhman *et al.* 1991) elevate the plasma levels of the μ-receptor agonist β-endorphin in male golden hamsters (*Mesocricetus auratus*).

Social conflict within a resident–intruder paradigm incorporates cues that would be predictive of sperm competition in the wild. For a rodent defending a territory in the wild, encountering, attacking and defeating a male intruder might be predictive of sperm competition risk since the intruder could have already copulated with a resident female. Hence, such experiences should trigger adaptive changes in ejaculate composition, possibly mediated by a reduction in the level of EOP secretion and a consequent increase in vas deferens contractility.

To test the hypothesis that species differences in the sensitivity of the vas deferens to opioid agonists are related to the extent to which sperm competition has been a selective pressure in each, two species from the *Peromyscus* genus were compared. *P. maniculatus* is probably the most polygynous species within the genus (Dewsbury 1981*a*). However, it is also known that, given the opportunity, *P. maniculatus* females will copulate with more than one male during a single oestrus cycle. Consequently, they often conceive litters of multiple paternity in the wild (Birdsall & Nash 1973) and in the laboratory (Dewsbury 1981*b*). Furthermore, the males that copulate most frequently with a polyandrous female are most likely to sire her offspring (Dewsbury 1985*a*). Therefore, although the mating system of *P. maniculatus* is effectively polygynous, female polyandry has been an important selective pressure for males of this species. In contrast, *Peromyscus californicus* pairs are highly, perhaps even exclusively, monogamous. There is no DNA evidence of extra-pair paternity in the wild (Ribble 1991) and both sexes avoid extra-pair copulation (Gubernick & Nordby 1993).

Antinociceptive responses to environmental stress (Kavaliers & Innes 1987; Kavaliers & Galea 1995; Kavaliers *et al.* 1998) and novelty (Kavaliers & Innes 1988) have been observed in *P. maniculatus*. However, the existence of social-conflict-induced, opioid-dependent analgesia has not yet been established in this species. Nevertheless, *P. maniculatus* males do form dominance relationships based on priority of residence (Dewsbury 1985*b*), relationships which are associated with opioid-dependent responses in laboratory mice and rats.

Since EOP secretion depends on social experiences that are predictive of sperm competition risk, and vas deferens contractility is affected by opioid agonists in many species, it is hypothesized that EOPs are involved in the adaptive regulation of ejaculate composition. Consequently, the vas deferens should be more sensitive to opioid agonists such as morphine in species with sperm competition, such as *P. maniculatus*, than in those without, such as *P. californicus*.

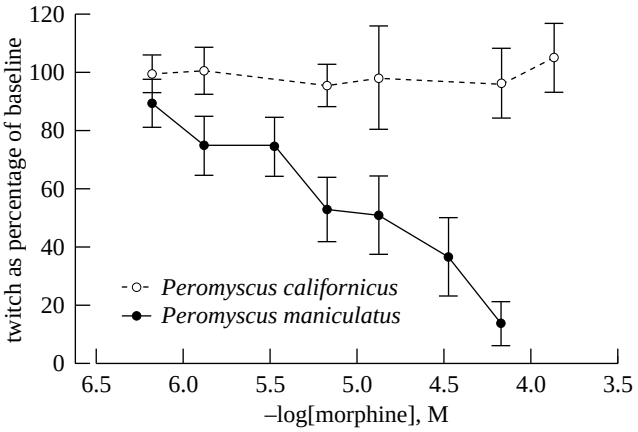

Figure 1. Effects of morphine on electrically stimulated twitch contractions of the vas deferens in *P. maniculatus* and *P. californicus*. Data are the means (±s.e.m) for six animals from each species.

2. METHODS

Sexually mature (2–12 months of age) male *Peromyscus californicus insignis* (44–52 g) and *Peromyscus maniculatus bairdii* (20–30 g) were used in the experiment (Peromyscus Genetic Stock Centre, University of South Carolina). Six animals from each species were sacrificed by cervical dislocation and the vasa deferentia, with the prostatic end sectioned as near as possible to the seminal vesicle, were dissected from fat and connective tissue. Each was gently pressed to expel the seminal contents before being mounted vertically in a 10 ml organ bath between two platinum ring electrodes (1 cm apart). The prostatic end of each vas deferens was tied with silk thread and connected to a Grass force displacement transducer, model FT 03 (Grass Instrument Co., Quincey, MA, USA).

Tissues were maintained at 37 °C in a modified (Mg^{2+}-free) Kreb's solution (118 mmol NaCl, 4.75 mmol KCl, 2.54 mmol $CaCl_2$, 1.03 mmol KH_2PO_4, 23 mmol $NaHCO_3$ and 11 mmol glucose) which was continuously bubbled with a mixture of 95% O_2 and 5% CO_2. A resting tension of *ca.* 200 mg was applied to each vas deferens and the tissues were allowed to equilibrate for 45 min before longitudinal contractions were recorded isometrically using a Grass model 7 polygraph.

To excite the intramural nerves, the electrical field stimulation parameters established by Henderson *et al.* (1972) were used (0.1 Hz, 1 ms, supramaximal voltage). Baseline twitch heights were established for each vas deferens. Then concentration–effect curves were generated cumulatively by adding morphine sulphate (Sabex) in 10–100 μl volumes from 0.5 mg ml^{-1}, 5 mg ml^{-1} and 50 mg ml^{-1} stock solutions. The pre-incubation time at each concentration was 20 min. The maximum twitch height was recorded once the tissues had equilibrated at each concentration and the responses of vasa deferentia from the same animal were averaged.

3. RESULTS

The dose–response curves for *P. californicus* and *P. maniculatus* are shown in figure 1. The maximum twitch

height for electrically evoked contractions of the *P. californicus* vas deferens was not inhibited by morphine at any dose. However, increasing doses of morphine progressively inhibited contractions of the *P. maniculatus* vas deferens.

4. DISCUSSION

The vas deferens of the laboratory mouse possesses μ-receptors (Hutchinson *et al*. 1975) and, consequently, electrically evoked contractions are inhibited by both morphine (Hughes *et al*. 1975) and β-endorphin (Sanchez-Blazquez *et al*. 1983). In the present experiment, the observed sensitivity of the isolated *P. maniculatus* vas deferens to the inhibitory effects of morphine *in vitro* indicates the presence of μ-receptors in this tissue. Consequently, in the intact animal the vas deferens should be able to respond to changes in the level of circulating endogenous μ-receptor agonists such as β-endorphin. Conversely, in the present experiment the isolated vas deferens of *P. californicus* was insensitive to morphine across the dose range tested, implying an absence of μ-receptors in this tissue. Lacking functional μ-receptors, the *P. californicus* vas deferens should be unable to respond to endogenous opioids that are agonists at this receptor subtype.

Previous research has shown that competitive interactions between males can affect the secretion of endogenous μ-receptor agonists such as β-endorphin (e.g. Rodgers & Hendrie 1983). Consequently, their levels in the peripheral circulation may provide an endocrine index of sperm competition risk that can directly affect the contractility of the vas deferens and, thus, facilitate the adaptive regulation of ejaculate volume and/or sperm concentration. Stimuli that tend to produce increases in EOP secretion will reduce the contractility of the vas deferens and inhibit sperm delivery. Conversely, stimuli that tend to produce decreases in EOP secretion will increase vas deferens contractility and stimulate sperm delivery.

Genotype-dependent responses of the vas deferens to an opioid agonist have been reported previously (Berti *et al.* 1978). However, in the present study the observed difference was predicted *a priori* based on evolutionary theory and findings from behavioural ecology. The reproductive success of *P. maniculatus* males depends on their ability to sire offspring in an environment in which females engage in polyandrous mating. Consequently, males must balance the need to inseminate more sperm when the risk of sperm competition is high against the non-trivial costs of ejaculate production. A potential mechanism by which this might be achieved involves the regulation of vas deferens contractility and, thus, sperm delivery by EOPs such as β-endorphin. For β-endorphin to regulate sperm delivery, the vas deferens must contain functional μ-receptors and the present experiment indicates that this is indeed the case for a species in which males face the selective pressures of sperm competition. In contrast, the experiment indicates that the vas deferens lacks functional μ-receptors in a species without sperm competition, *P. californicus*, in which the ability to regulate sperm delivery in response to changes in the social environment confers no selective advantage. In light of these findings, the hypothesis that adaptive regulation of sperm competition depends on EOP-regulated changes in the contractility of the vas deferens warrants further investigation.

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