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κ-Opioid receptor-mediated analgesia: hotplate temperature and sex differences

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Abstract

The present experiment was designed to investigate the dose–effect and time–effect functions of the κ − opioid receptor agonist [5α,7α,8β)-(−)-*N*-Methyl-*N*-[7-(1-pyrrolidinyl)-1-oxaspiro(4,5)dec-8-yl]benzeneacetamide] (U69,593) in intact and gonadectomized male and female rats as a function of hotplate temperature (45°C or 51°C). At 45°C baseline lick latencies were longer in female rats than in male rats. Lick latencies increased dose-dependently in all subjects except intact female rats. U69,593 increased lick latencies as a function of time since its administration in all subjects. At 51°C baseline lick latencies did not differ between groups and they increased dose-dependently in all subjects. The effectiveness of U69,593 decreased as a function of time since its administration, but not in castrated male rats. These observations suggest that gonadal hormones could play a role in modulating the behavioral effects of U69,593 when subjects are tested at different hotplate temperatures.

Keywords: Opioid analgesia; U69,593; Hotplate; Sex differences; Lick latency; (Rat)

1. Introduction

Organismic variables affect opioid analgesia (e.g. Bodnar et al., 1988). It has been observed that the behavioral effects of μ-opioid receptor agonists in male rats exceed those observed in female rats (Cicero et al., 1996, 1997; Keppler et al., 1989, 1991), but others have failed to report these sex-dependent effects (Bartok and Craft, 1997). It is also not immediately obvious how manipulation of gonadal hormones in adulthood alters the behavioral response to noxious stimulation. Different authors have reported potentiation or attenuation of antinociception by μ-opioid receptor agonists following such manipulations (Ali et al., 1995; Islam et al., 1993; Kepler et al., 1989, 1991). We have recently reported that hotplate temperature may play a role in whether or not sex differences are observed (Haaren et al., submitted). This experiment was conducted because it had previously been proposed that cutaneous nociceptors, classified as either myelinated Aδ nociceptors or unmyelinated C-fiber nociceptors (Burgess and Perl, 1973), respond differently dependent upon the intensity of the noxious stimulus (Yeomans et al., 1996; Yeomans and Proudfit, 1996). C fibers fire predominantly when heating occurs at a low rate (0.9°C/s) which corresponds to a skin temperature of approximately 43°C to 45°C. Aδ fibers fire mostly when heating occurs at a high rate (6.5°C/s), which corresponds to a skin temperature of about 49°C to 57°C. It has been reported that morphine preferentially alters responses mediated by the activation of C fiber nociceptors (Jurna and Heinz, 1979; Yeomans and Proudfit, 1996; Yeomans et al., 1996). In our experiment, acute morphine administration did not affect lick latencies sex dependently at a hotplate temperature of 45°C. The effects of morphine decreased as a function of the time since its administration in all subjects when they were exposed to the 45°C hotplate. At 51°C, lick latencies were longer following morphine administration in ovariectomized female rats than in other subjects. Peak effects of morphine were observed 30 min after its administration in ovariectomized female rats, but at least 60 min following its administration in other subjects.

Evidence has also been presented to suggest that organismic variables may affect κ-opioid receptor-mediated

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analgesia. In humans, Gear et al. (1996a,b) have reported that the κ-opioids nalbuphine and butorphanol produced greater analgesia in women than in men following dental surgery. Others have shown in laboratory animals that the κ-opioid agonist U50,488 produced greater antinociception in male deer mice than in female deer mice (Kavaliers and Innis, 1987). More recently, Bartok and Craft (1997) did not observe sex differences following U69,593 administration on a hot plate maintained at 52°C, but they did observe sex differences in a tail withdrawal assay. In a different context, Craft et al. (1998) have also reported that male rats are more sensitive than female rats to the discriminative stimulus and diuretic effects of U69,593.

The present experiment was designed to further delineate the activational effects of gonadal hormones on nociception following the administration of U69,593. We hypothesized that gonadal status might differentially influence the efficacy of U69,593 at low and high rates of thermal stimulation. To test this hypothesis, we determined U69,593's dose–effect function (vehicle, 0.03, 0.10, 0.30, 0.56 mg/kg) in intact and gonadectomized male and female rats exposed to hotplate temperatures of 45°C (low heating rate) and 51°C (high heating rate). In addition, a single *effective* dose of U69,593 was selected for each hotplate temperature and used to establish U69,593's time–effect function in intact and gonadectomized male and female rats. All tests were conducted within subjects to limit the number of subjects required to conduct the experiment.

2. Materials and methods

2.1. Subjects and surgery

Eighteen male and 18 female Sprague–Dawley rats were obtained from Harlan Sprague Dawley (Indianapolis, IN) when they were approximately 60 days old. They were housed in groups of three and maintained on a 12 h light–dark cycle (lights on at 0700 h). Rat chow and water were always available and the vivarium was maintained at a constant temperature (21°C). Nine male rats were orchidectomized, nine female rats were ovariectomized. The other male and female rats received sham surgery. Orchidectomy was accomplished by removal of the testes and testicular fat through a 2-cm midscrotal incision. Ovariectomy was inflicted by removal of the ovaries and ovarian

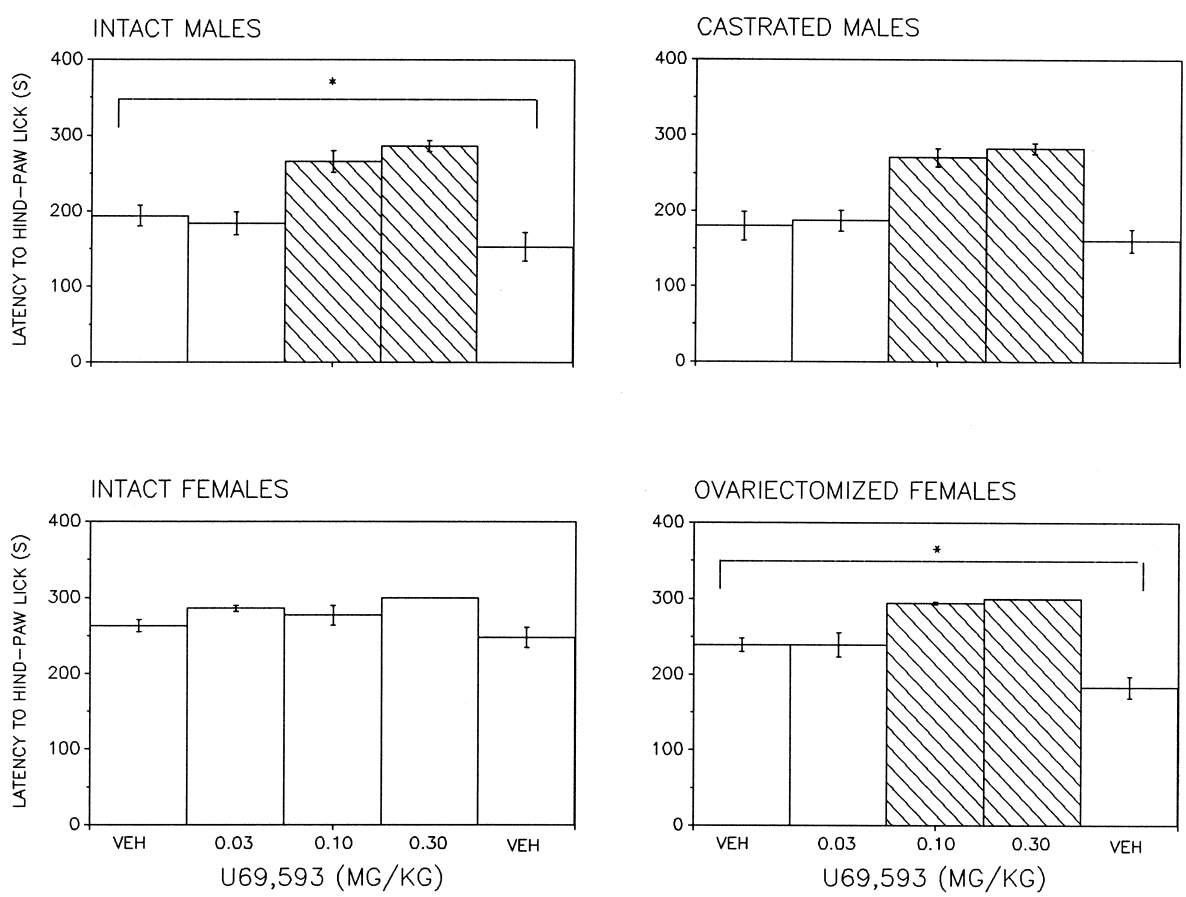

Fig. 1. The effects of different doses of U69,593 (vehicle, 0.03, 0.10 and 0.30 mg/kg, S.C., −30 min) on hind paw lick latency (s, ±1 S.E.M.) in intact male rats (upper left-hand panel), castrated male rats (upper right-hand panel), intact female rats (lower left-hand panel) and ovariectomized female rats (lower right-hand panel) when subjects were exposed to a hotplate maintained at 45°C. Maximum test duration was 300 s. Vertically hatched bars indicate behavioral effects that were different from those observed during vehicle administration.

fat with a dorsal incision. During sham surgery the testes and ovaries were exposed but not removed. The subjects were allowed 10 days to recover from surgery, during which they were handled every day.

2.2. Apparatus

The hotplate tests were conducted during the subjects' light period on an IITC Export model 35-D Analgesiameter in a dimly lit room. A Plexiglas box without a lid (25 cm high) enclosed the hotplate. Latency to the first hind-paw lick or escape from the cylinder (which rarely occurred) was used as the behavioral end-point. Sessions were terminated after 300 s when tests were conducted at 45°C or after 30 s when tests were conducted at 51°C to avoid tissue damage. The maximum latency was assigned to any subject that failed to emit the hind-paw lick or to escape.

2.3. Drugs

[5α,7α,8β)-(−)-*N*-Methyl-*N*-[7-(1-pyrrolidinyl)-1-oxaspiro(4,5)dec-8-yl]benzeneacetamide] (U69,593) was obtained from the National Institute on Drug Abuse (Research Triangle Park, NC) and prepared daily in the appropriate concentrations. U69,593 was dissolved in 25% propylene glycol and subcutaneously (s.c.) administered in a volume of 1 ml/kg bodyweight (cf. Schenk et al., 1999). Drug tests were conducted at least three days apart when the dose–effect function was established.

2.4. Procedure

All subjects participated in the following four experiments.

2.4.1. Experiment 1. Determination of the U69,593 dose–effect curve at a hotplate temperature of 45°C

First, hind-paw lick latency was established 30 min following s.c. vehicle injection (25% propylene glycol) during three consecutive daily sessions. All subjects were then tested once with 0.10, 0.30 and 0.03 mg/kg U69,593. To conclude this experimental condition, subjects were again exposed to the hotplate 30 min following vehicle administration.

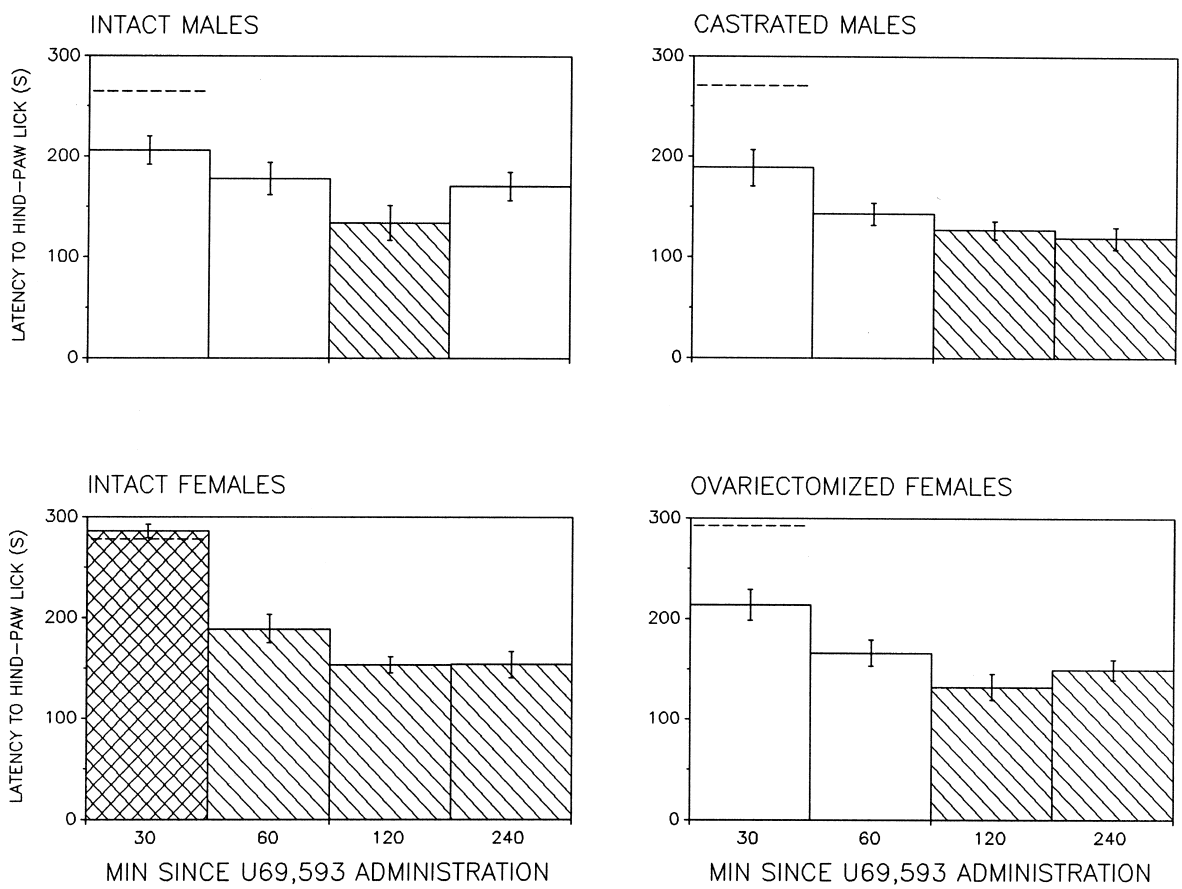

Fig. 2. The effects of 0.10 mg/kg U69,593 on hind paw lick latency (s, ±1 S.E.M.) observed 30, 60, 120 and 240 min following its administration in intact male rats (upper left-hand panel), castrated male rats (upper right-hand panel), intact female rats (lower left-hand panel) and ovariectomized female rats (lower right-hand panel). The hot plate temperature was 45°C and the maximum test duration was 300 s. Horizontal dotted lines in each panel indicate the behavioral effects of 0.10 mg/kg U69,593 observed in the previous experimental condition. Vertically hatched bars indicate behavioral effects that were different from those observed 30 min following the administration of U69,593.

2.4.2. Experiment 2. Determination of the U69,593 time–effect curve at a hotplate temperature of 45°C

All subjects were s.c. injected with 0.10 mg/kg U69,593 and hind paw lick latencies were recorded 30, 60, 120 and 240 min thereafter. This particular dose was selected because it was the lowest effective dose in the previous experimental condition.

2.4.3. Experiment 3. Determination of the U69,593 dose–effect curve at a hotplate temperature of 51°C

On three different occasions, hind-paw lick latencies were established 30 min following vehicle injection (25% propylene glycol solution). All subjects were then tested once following an injection of 0.03, 0.10, 0.30 and 0.56 mg/kg U69,593. To conclude this experimental condition, subjects were again exposed to the hotplate 30 min following s.c. vehicle administration.

2.4.4. Experiment 4. Determination of the U69,593 time–effect curve at a hotplate temperature of 51°C

All subjects were s.c. injected with 0.30 mg/kg U69,593 and hind paw lick latencies were recorded 30, 60, 120 and 240 min thereafter. This dose was the lowest effective dose in the previous experimental condition.

2.5. Data analyses

Dose–effect and time–effect functions of U69,593 were evaluated with a two-factor repeated measures analysis of variance (ANOVA) involving the factors GROUP (intact, castrated male rats and intact, ovariectomized female rats and DOSE (different doses of U69,593) or TIME (30, 60, 120 and 240 min following administration). ANOVA with GROUP and TIME (before–after) was used to compare the behavioral effects of vehicle administration prior to and following the administration of U69,593. Duncan's multiple-range tests were used where appropriate. *P*-values less than or equal to 0.05 were considered significant.

3. Results

Fig. 1 shows the effects of different doses of U69,593 (vehicle, 0.03, 0.10 and 0.30 mg/kg, s.c., −30 min) on hind paw lick latency (s, ±1 S.E.M.) in intact male rats (upper left-hand panel), castrated male rats (upper right-hand panel), intact female rats (lower left-hand panel) and ovariectomized female rats (lower right-hand panel) when

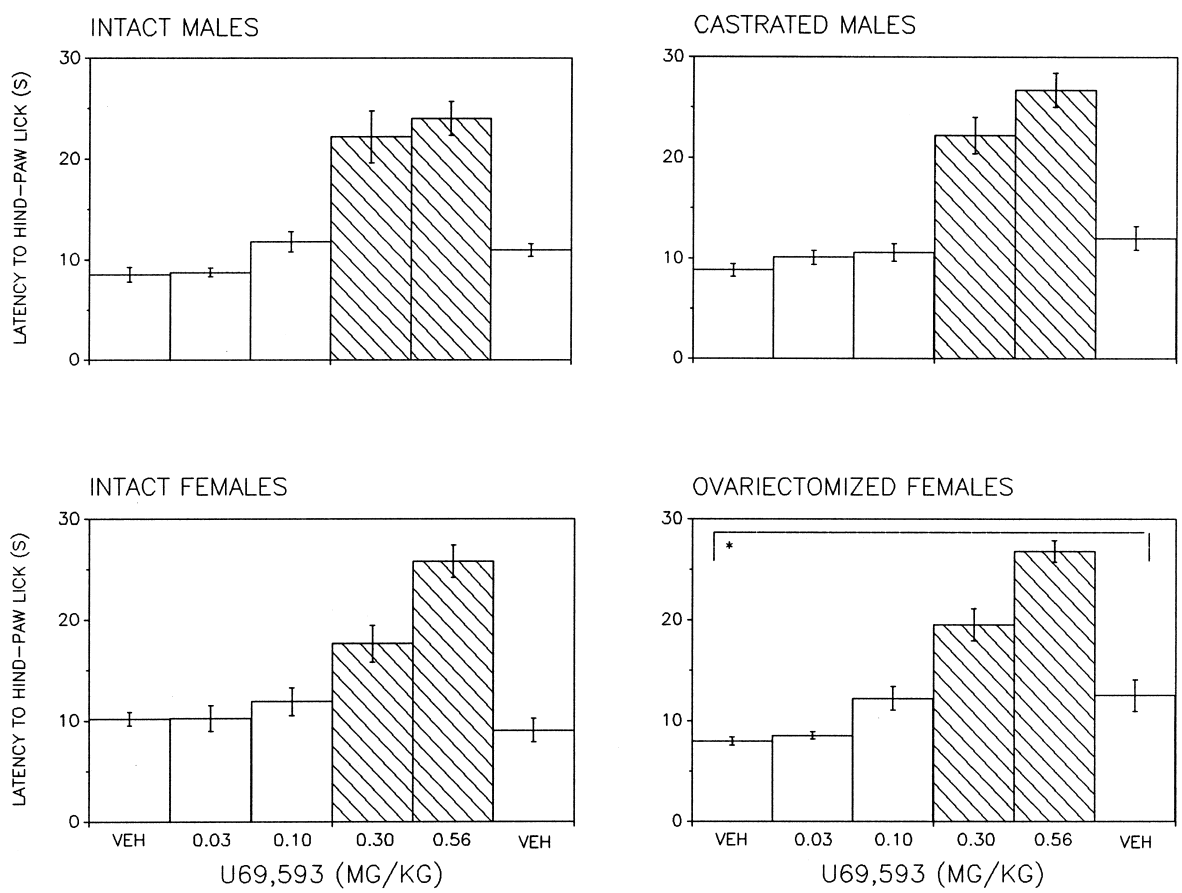

Fig. 3. The effects of different doses of U69,593 (vehicle, 0.03, 0.10, 0.30 and 0.56 mg/kg, S.C., −30 min) on hind paw lick latency (s, ±1 S.E.M.) in intact male rats (upper left-hand panel), castrated male rats (upper right-hand panel), intact female rats (lower left-hand panel) and ovariectomized female rats (lower right-hand panel) when these subjects were exposed to a hotplate maintained at 51°C. Maximum test duration was 30 s. Vertically hatched bars indicate behavioral effects following the administration of U69,593 that were different from those observed during vehicle administration.

these subjects were exposed to a hotplate maintained at 45°C.

Following vehicle administration, hind paw lick latencies averaged 194, 179, 263 and 239 s for intact and castrated male rats and intact and ovariectomized female rats, respectively. Latencies were longer in female rats than in male rats. Lick latencies increased dose-dependently following the administration of U69,593 (Dose, $F(3,93) = 31.12$, $P < 0.01$). Group differences were also observed (Group, $F(3,31) = 6.70$, $P < 0.01$) as was a Group by Dose interaction ($F(9, 93) = 2.70$, $P < 0.01$). Post-hoc analyses revealed that following administration of 0.10 and 0.30 mg/kg U69,593 lick latencies were longer in all rats except intact female rats, as compared to latencies observed after vehicle administration. A comparison of hind paw lick latencies assessed prior to and following the examination of the dose–effect function, revealed that post-assessment latencies were shorter in all rats, but significantly shorter in intact male rats and ovariectomized female rats (Group, $F(3,31) = 6.15$, $P < 0.01$, Before–After, $F(1, 31) = 12.69$, $P < 0.01$).

Fig. 2 shows the effects of U69,593 (0.10 mg/kg) on hind paw lick latencies observed 30, 60, 120 and 240 min following its administration. The horizontal dotted lines in each panel indicate the behavioral effects of the same dose of U69,593 as they were observed in the preceding experimental condition.

The effectiveness of U69,593 decreased as a function of the time since its administration in all subjects (Time, $F(3,31) = 22.04$, $P < 0.01$). Group differences were also observed (Group, $F(3,31) = 3.69$, $P < 0.05$), as was a significant Group by Time interaction ($F(9,93) = 1.52$, $P < 0.05$). Thirty minutes after the administration of 0.10 mg/kg U69,593 lick latencies were significantly longer in intact female rats than in any of the other groups of subjects (crosshatched bars). As can be seen in Fig. 2, a dose of 0.10 mg/kg U69,593 increased lick latencies for up to 60 min in intact and castrated male rats and ovariectomized female rats.

Fig. 3 shows the effects of different doses of U69,593 (vehicle, 0.03, 0.10, 0.30 and 0.56 mg/kg) on lick latency when subjects were exposed to a hotplate maintained at 51°C.

Hind paw lick latencies following vehicle administration averaged 8.5, 8.8, 10.2 and 8.3 s for intact and castrated male rats and intact and ovariectomized female

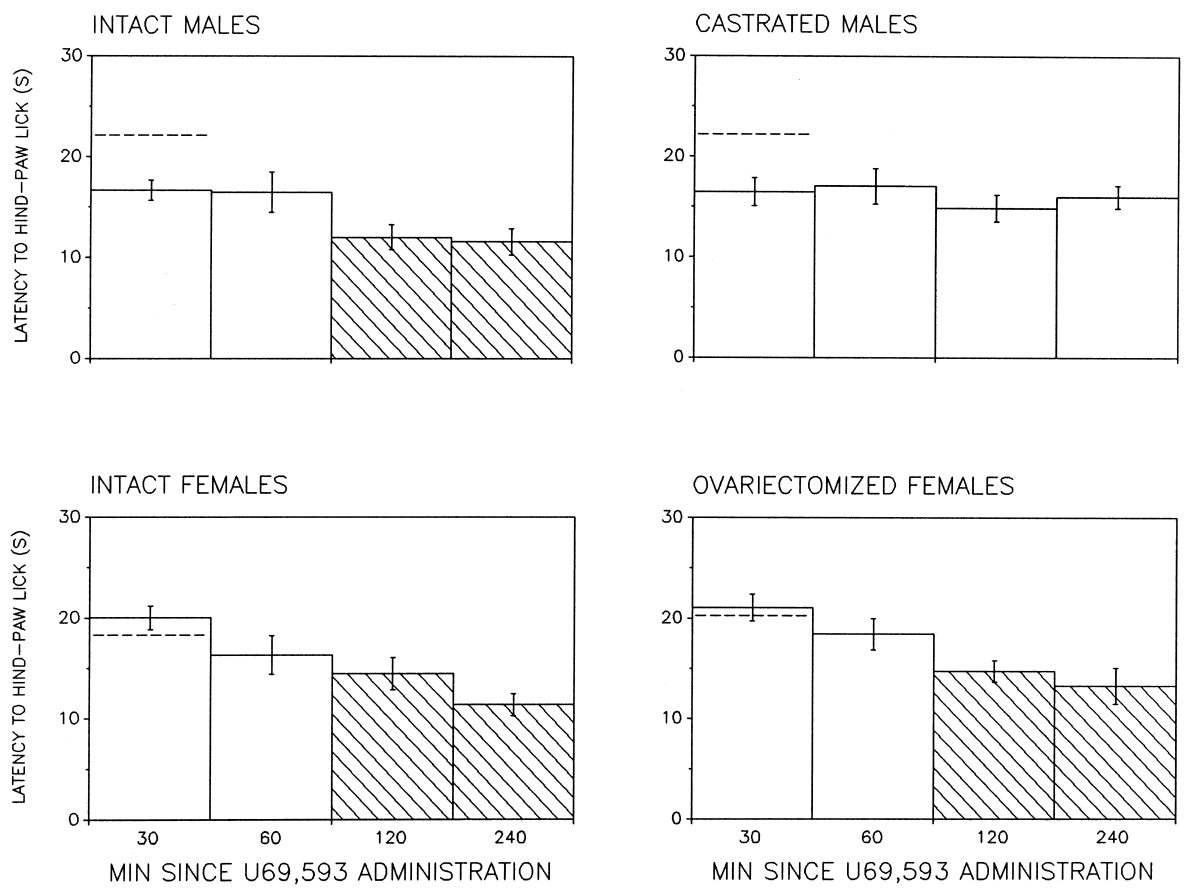

Fig. 4. The effects of 0.30 mg/kg U69,593 on hind paw lick latency (s, ±1 S.E.M.) observed 30, 60, 120 and 240 min following its administration in intact male rats (upper left-hand panel), castrated male rats (upper right-hand panel), intact female rats (lower left-hand panel) and ovariectomized female rats (lower right-hand panel). The hotplate temperature was 51°C and the maximum test duration was 30 s. Horizontal dotted lines in each panel indicate the behavioral effects of 0.30 mg/kg U69,593 observed in the previous experimental condition. Vertically hatched bars indicate behavioral effects that were different from those observed 30 min following its administration.

rats, respectively (no differences between groups). ANOVA indicated that hind paw lick latencies increased as a function of the dose of U69,593 (Dose, $F(4, 31) = 109.68$, $P < 0.01$), but did not differ between groups of subjects (Group, $F(3,31) = 0.07$, n.s.). Hind paw lick latencies after the administration of 0.30 and 0.56 mg/kg U69,593 exceeded those observed after vehicle administration. A comparison of hind paw lick latencies prior to and following the assessment of the dose–effect function of U69,593 showed that post-assessment latencies were longer for ovariectomized female rats, but not for any of the other subjects.

Fig. 4 shows the effects of 0.30 mg/kg U69,593 on hind paw lick latencies observed 30, 60, 120 and 240 min following its administration. The hotplate was maintained at 51°C and the maximum test duration was 30 s. The horizontal dotted lines in each panel indicate the behavioral effects of 0.30 mg/kg U69,593 as they had been observed in the preceding experimental condition.

The effectiveness of U69,593 decreased as a function of the time since its administration in all groups of subjects (Time, $F(3,31) = 14.59$, $P < 0.01$). Group differences were not observed, even though peak effects decreased over time in intact male rats and intact and ovariectomized female rats, but not in castrated male rats.

4. Discussion

The present experiment was designed to investigate the dose–effect and time–effect functions of U69,593 in intact and gonadectomized male and female rats as a function of hotplate temperature. Hotplate temperature was manipulated because it has previously been suggested that pain sensations elicited by different thermal stimuli may preferentially recruit Aδ or C fibers (Yeomans and Proudfit, 1996; Yeomans et al., 1996). Lick latencies increased dose-dependently in all groups of subjects except intact female rats, when subjects were exposed to the hotplate at 45°C. This, however, may have been because of sex differences in baseline lick latencies (longer in females than in males) and/or ceiling effects due to the time limit on test duration. A dose of 0.10 mg/kg U69,593 increased lick latencies as a function of time since its administration in all subjects. Thirty minutes after administration, its effects were more pronounced in intact female rats than in any of the other groups of subjects, but they did not last as long. As such these observations confirm those reported by Bartok and Craft (1997) who showed that the peak effects of U69,593 on tail withdrawal tended to occur earlier in females than in males, but they are hard to reconcile with those of Craft et al. (1998) who showed that the ED_{50} for the discriminative stimulus effects of U69,593 was approximately threefold higher in female rats than in male rats (0.074 vs. 0.025 after training at 0.13 mg/kg). But then, maybe the analgesic and discriminative stimulus effects of U69,593 are not necessarily correlated.

When subjects were exposed to a hotplate maintained at 51°C, hind paw lick latencies increased dose-dependently in all groups of subjects. U69,593 increased lick latencies as a function of the time since its administration in intact male and female rats and ovariectomized female rats, but not in castrated male rats. The latter observations suggest that gonadal hormones may interact with environmental variables to modulate the analgesic effects of U69,593.

Lick latencies were much shorter when subjects were exposed to a hotplate maintained at 51°C than when they were exposed to a hotplate maintained at 45°C. Sex differences were not observed when subjects were tested at a hotplate temperature of 51°C, but lick latencies were much shorter in male rats than in female rats when subjects were tested at 45°C. These sex differences in lick latencies on the 45°C hotplate confirm results observed previously in our laboratory (Haaren et al., submitted; Tucker et al., submitted). It appears that testing at 45°C allows for more interindividual variability than testing at 51°C, one of the reasons why investigators have preferred to test at the higher temperature (Plone et al., 1996). Even though it may be desirable to limit interindividual variability as much as possible, doing so may also work to obscure otherwise important experimental information.

The present experiment differed from most other experiments designed to evaluate the effects of opioid administration on hotplate nociception in one important way. Whereas most other studies employed between-group designs to evaluate these effects, the present experiments employed a within-subjects design to do the same. Previously, we showed that lick latencies could easily be replicated between experimental conditions lending credence to the suggestion that repeated testing did not result in sensitization as a function of repeated exposure to the hotplate (Plone et al., 1996) or tolerance as a function of repeated morphine administration. The latter, of course, would have been unlikely to occur anyway given that the development of morphine tolerance appears to depend upon frequent administration of high doses (i.e. two weeks, twice daily, 10–20 mg/kg; Craft et al., 1999). In the present experiment vehicle lick latencies prior to and following assessment of the dose–effect curve on the 45°C and 51°C hotplate could only consistently be replicated in intact female rats. Changes in latencies were observed in all other groups of subjects, suggesting that repeated hotplate exposure in combination with the administration of U69,593 may differentially affect lick latencies dependent upon the subject's gonadal status. It remains to be determined what variables in particular could be responsible for these observations.

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