Physiology & Behavior, Vol. 64, No. 4, pp. 445–450, 1998

Printed in the U.S.A.
0031-9384/98 $19.00 + .00

ELSEVIER

PII S0031-9384(98)00054–7

Effect of American Ginseng *(Panax quinquefolium)* on Male Copulatory Behavior in the Rat

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Received 10 December 1997; Accepted 12 January 1998

MURPHY, L. L., R. S. CADENA, D. CHÁVEZ AND J. S. FERRARO. *Effect of American Ginseng (Panax quinquefolium) on male copulatory behavior in the rat.* **64**(4) 445–450, 1998.—The effects of American ginseng (*Panax quinquefolium*) on male rat copulatory behavior were investigated. Adult Sprague-Dawley rats were administered either 10, 50 or 100 mg/kg of *Panax quinquefolium* or a sesame oil vehicle per os *(p.o.)* for 28 days and copulatory behavior parameters were measured. Ginseng-treated male rats demonstrated a significant decrease in mount, intromission and ejaculation latencies compared to vehicle controls. Hormone analyses revealed no difference in plasma luteinizing hormone or testosterone levels between ginseng- and vehicle-treated animals; however, plasma prolactin levels were significantly reduced by all doses of ginseng tested. When male rats were treated with the 100 mg/kg dose of ginseng for 1, 14 or 28 days, mount and intromission latencies were significantly reduced at 14 and 28 days of daily ginseng treatment, whereas ejaculation latency was significantly reduced after 1 day of ginseng treatment when compared to vehicle controls. Plasma prolactin levels were also significantly decreased after 14 and 28 days of daily ginseng administration. There were no differences in body weight or in testes, seminal vesicle, anterior pituitary or spleen weights between ginseng- and vehicle-treated rats. These results demonstrate that *P. quinquefolium* significantly facilitates male copulatory behavior. The reduction in plasma prolactin levels suggests that ginseng-induced alterations in dopaminergic neurotransmission may play a role in the ability of *P. quinquefolium* to stimulate copulatory behavior in the male rat. © 1998 Elsevier Science Inc.

Panax quinquefolium Ginseng Copulatory behavior Male rat Testosterone Prolactin

ASIAN ginseng (*Panax ginseng* C.A. Meyer) and its close relative, American ginseng (*Panax quinquefolium* L.), are perennial aromatic herbs that are widely used in Oriental medicine (2,10,15). The root or root extract of *P. ginseng* has been demonstrated to induce vasodilation (6,29), inhibit platelet aggregation (14,27), enhance learning and memory (1,22), produce anxiolytic effects (4,5), and facilitate male rat copulatory behavior (12). Much less is known concerning the physiological effects of *P. quinquefolium*. An extract of *P. quinquefolium* root has been shown to induce hypoglycemia in normal and alloxan-treated mice (21).

Recent studies have investigated the physiological effects of ginsenoside saponins, the biologically active constituents of ginseng (25), and determined that specific ginsenosides can elicit significant effects on nitric oxide synthesis (7), acetylcholine-induced catecholamine secretion (26), maternal aggression (31), and glycemic activity (20). Although over 30 different ginsenosides have been identified overall (25,26), the ginsenoside content between different strains of ginseng is vastly different (2), suggesting that distinct ginseng strains may produce different physiological effects.

An extract of *P. ginseng* administered to male rats has been shown to significantly influence their copulatory behavior (12). Indeed, the latency to ejaculate and the postejaculatory refractory period were dramatically reduced in the ginseng-treated rats. The current study was undertaken to determine the effects of a wide dose range of *P. quinquefolium* on male copulatory behavior in rats. Furthermore, ginseng effects on hormone secretion were assessed in order to address the possible mode of action of *P. quinquefolium* in its ability to enhance male copulatory behavior.

METHOD

Animals

Adult male and female Sprague-Dawley rats were obtained from Harlan Sprague-Dawley, Inc. (Indianapolis, IN, USA) and housed in environmentally controlled animal quarters illuminated by fluorescent lighting 12 h each day (0600–1800 hours). Teklad

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laboratory chow and water were provided ad libitum. Four weeks prior to testing, female rats were ovariectomized and immediately implanted with a 5-mm Silastic capsule (3.175 × 2.413 mm) filled with 17β-estradiol (Sigma). Approximately 5 h before an experiment, females were injected subutaneously (s.c.) with 0.5 mg of progesterone (Sigma). Receptivity of each female rat was tested just prior to the behavior studies using spare male rats, and only those females exhibiting the lordotic posture were utilized. Prior to treatment, male rats were subjected to two preliminary mating tests of 30-min duration. Only animals that exhibited mounting and intromission or ejaculation were used in the following study. All experimental protocols were performed in accordance with Public Health Service regulations.

Treatment

An amount of powdered American ginseng root (*P. quinquefolium*), obtained from Sigma, was added to a sesame oil vehicle to achieve the desired concentration of either 10, 50, or 100 mg of ginseng/mL of vehicle. Male rats (approximately 75 days old) received ginseng or sesame oil alone (control group) in a volume of 0.1 mL/100 g of body weight by oral administration as has been previously described (19). Briefly, the proper dose of ginseng emulsion or oil was drawn up into a 1-mL tuberculin syringe fitted with a blunted 1.9 cm, 19-gauge needle, the tip of the needle was gently placed in the corner of the animal's mouth, and the contents of the syringe were slowly administered (19). Because the animals found the ginseng palatable, gastric intubation was not necessary. Animals were fed ginseng or vehicle between 0900 and 1000 hours each day for 28 days. In a second study, another group of male rats was fed 100 mg/kg of ginseng for either 1, 14 or 28 days. The control oil-fed animals were treated for 28 days only.

Experimental Protocol

All experiments were performed between 1830 and 2130 hours on the last day of treatment (on the same day of treatment in the 1-day ginseng group), starting 30 min after lights were turned off, in an area dimly lit by 20-W red bulbs. Male rats were placed individually in bedding-lined aquaria and allowed to acclimate to the test chamber for 5 min before the introduction of a sexually receptive female. The following copulatory behavior parameters were measured as described earlier (18): mount latency—time from introduction of the female until the first mount with pelvic thrusting; intromission latency—time from introduction of the female until first mount with pelvic thrusting and vaginal penetration; ejaculation latency—time from the first intromission until ejaculation; and postejaculatory interval—time from ejaculation until next intromission. Copulatory testing was terminated either after the end of the 30-min testing period or after the postejaculatory interval was recorded.

Male rats were sacrificed by decapitation approximately 24 h after their last ginseng/oil dosage for blood and tissue collection (approximately 14 h post-behavior testing). Trunk blood was collected into centrifuge tubes containing 6% EDTA (anticoagulant) and plasma was prepared and stored frozen for the analysis of luteinizing hormone (LH), prolactin, and testosterone.

Radioimmunoassays

Plasma LH and prolactin concentrations were determined in duplicate in single double-antibody radioimmunoassays using kits supplied by the National Institute of Diabetes and Digestive and Kidney Diseases (NIADDK) as described previously (18). Testosterone levels were measured in duplicate using a double antibody radioimmunoassay kit from Diagnostic Products Corporation (Los Angeles, CA, USA) according to manufacturer's instructions. The sensitivities of the LH, prolactin, and testosterone assays were 0.024, 0.0195 and 0.025 ng/mL, respectively, and intra-assay coefficients of variation were 5.9, 4.8 and 3.2%, respectively. Peptide hormone results were expressed in terms of the RP-3 reference preparations.

Data Analysis

Each experimental group consisted of twelve animals and data are presented as means ± SEM. Hormone data were analyzed using two-way ANOVA to determine statistical significance of differences between treatment means. Copulatory behavior latency periods in vehicle control versus ginseng-treated male rats were evaluated nonparametrically with Fisher's exact probability test. In all cases, $p < 0.05$ was taken as the level of significance.

RESULTS

Copulatory Behavior

In the first set of experiments, copulatory behavior was examined in male rats treated with either 10, 50 or 100 mg/kg of ginseng for 28 days. All doses of ginseng tested dramatically reduced the latency to mount ($p < 0.05$) when compared to vehicle controls (Fig. 1A). Although there was a trend toward a decrease in intromission latency in ginseng-treated animals, only the 100 mg/kg dose of ginseng was significant when compared to vehicle-treated rats (Fig. 1B). The latency to ejaculate was also significantly reduced ($p < 0.05$) in ginseng-treated animals in all ginseng treatment groups (Fig. 1C). The post-ejaculatory interval was not different in ginseng- versus vehicle-treated animals (Fig. 1D).

In the next study, copulatory behavior was examined in male rats treated with the 100 mg/kg dose of ginseng for either 1, 14 or 28 days. Mount latency was significantly reduced ($p < 0.05$) after either 14 or 28 days of ginseng treatment when compared to vehicle-treated controls (Fig. 2A). There was a similar reduction in intromission latency in animals administered ginseng for either 14 or 28 days (Fig. 2B). There appeared to be a trend toward a decrease in both mount and intromission latencies after an acute dose of ginseng, however the decrease was not statistically significant. There was, however, a significant reduction in ejaculation latency following acute ginseng treatment (Fig. 2C). Although ejaculation latency was also significantly decreased after 28 days of ginseng administration ($p < 0.05$), the reduction in ejaculation latency following 14 d of ginseng treatment was not statistically significant. Again, the postejaculatory interval was unaffected in ginseng-treated animals when compared to vehicle controls (Fig. 2D).

Hormone Levels/Tissue Weights

Neither the ginseng dose nor the duration of ginseng treatment significantly affected plasma levels of LH or testosterone when compared to vehicle controls (data not shown). However, plasma prolactin levels were significantly decreased by all doses of ginseng tested when compared to vehicle-treated controls (Fig. 3A). Although the decrease in prolactin levels was not observed after 1 day of treatment, following 14 or 28 days of ginseng treatment, prolactin levels were significantly reduced relative to vehicle controls (Fig. 3B).

None of the ginseng doses appeared to affect body weight or testes weight, nor did ginseng influence seminal vesicle, anterior pituitary or spleen weights (data not shown). Similarly, the duration of ginseng exposure did not alter either body or tissue weights.

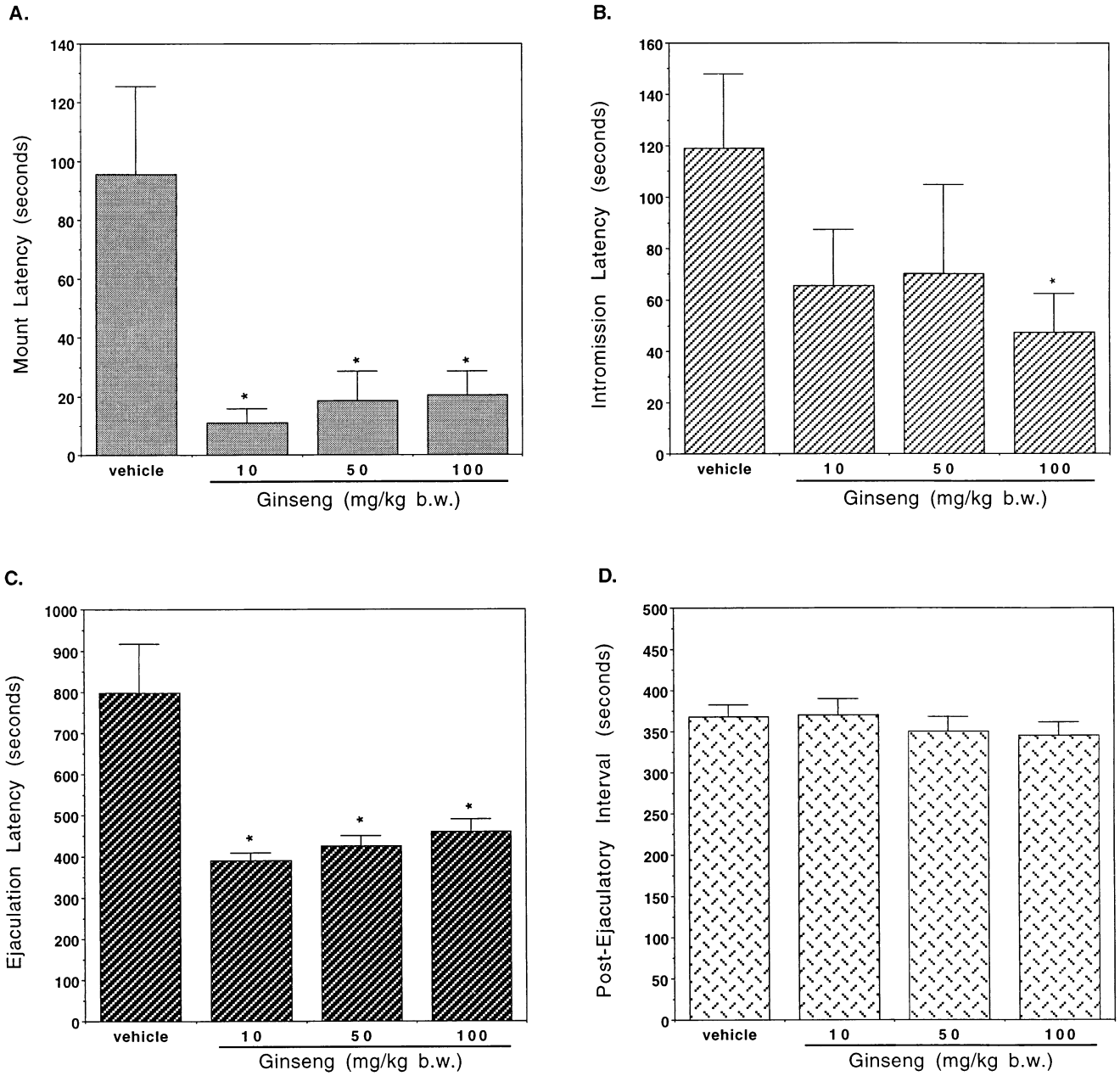

FIG. 1. Effect of ginseng doses on mount latency (A), intromission latency (B), ejaculation latency (C) and the postejaculatory interval (D) in male rats treated with ginseng or vehicle daily for 28 days. Results are expressed as mean ± SEM with twelve animals per treatment group. Asterisks denote significance at $p < 0.05$ when compared to respective vehicle group.

DISCUSSION

The present study provides new information concerning the ability of *P. quinquefolium* to enhance male copulatory behavior in rats. Although one earlier study revealed that *P. ginseng* was also able to stimulate male rat copulatory behavior (12), interesting differences in the two studies could be found. In the current study, *P. quinquefolium* significantly enhanced male sexual arousal, as evidenced by reduced latency to mount the female, and increased copulatory performance, as indicated by the ability of ginseng treatment to reduce intromission and ejaculation latencies. In contrast, treatment with *P. ginseng* significantly reduced ejaculation latency but did not affect either mount or intromission latency (12). Interestingly, *P. ginseng* also decreased the refractory period or postejaculatory interval in male rats. Treatment differences alone could account for the results obtained with the different ginsengs; *P. ginseng* extract (20 mg/kg of body weight) was given subcutaneously for 5 days, whereas powdered *P. quinquefolium* root (10, 50 or 100 mg/kg of body weight) was given orally for 1, 14 or 28 days. It is noteworthy that *P. quinquefolium* (100 mg/kg) decreased ejaculation latency after either 1 or 28 days of daily administration, suggesting that tolerance development may not be a factor in the copulatory behavioral effects of ginseng. Although

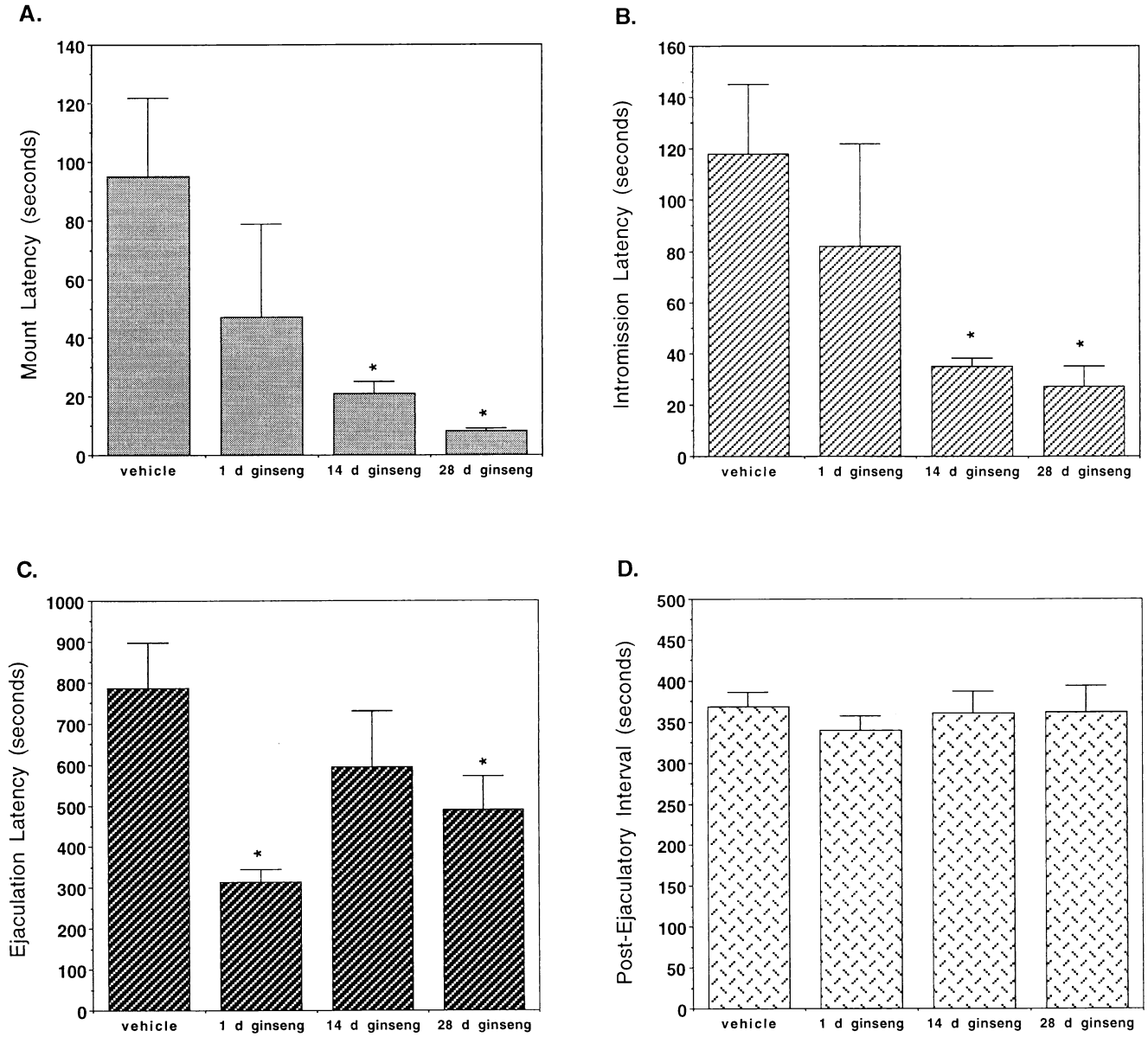

FIG. 2. Effect of ginseng (100 mg/kg) administered daily for 1, 14 or 28 days on mount latency (A), intromission latency (B), ejaculation latency (C), or the postejaculatory interval (D) in male rats. Results are expressed as mean ± SEM with twelve animals per treatment group. Asterisks denote significance at $p < 0.05$ when compared to respective vehicle group.

it is likely that the unique chemical composition of the two ginsengs (2) and, in particular, the concentration of specific ginsenosides dictate the behavioral response to ginseng preparations, it is currently unknown which ginsenoside(s) may be responsible for the effects of *P. quinquefolium* and *P. ginseng* on male copulatory behavior.

Early studies reported that chronic administration of *P. ginseng* produced a dose-related increase in serum testosterone levels in male rats (9), suggesting that testosterone might mediate the heightened copulatory behavior in ginseng-treated animals. Indeed, facilitation of copulatory behavior following acute testosterone injection has been demonstrated in sexually-experienced male rats (17). In the current study, *P. quinquefolium*-treated male rats did not exhibit changes in plasma LH or testosterone levels, or in androgen-dependent tissue weights, following acute or chronic ginseng exposure. Although hormone levels were measured approximately 24 h after the last treatment dose, which might have prevented the detection of an acute rise in testosterone induced by ginseng, it is unlikely that testosterone mediates the behavioral effects of *P. quinquefolium*. First, copulatory behavior testing took place approximately 8–10 h after the last treatment dose of ginseng, whereas an acute testosterone injection stimulated male copulatory behavior within 1 h of injection (17). Second, it has been reported that the average plasma testosterone levels in adult male rats exceed those required for quantitative maintenance of copulatory behavior in castrated rats (8). Thus, a transient rise in testosterone levels would not be expected to exert any effects on copulatory behavior.

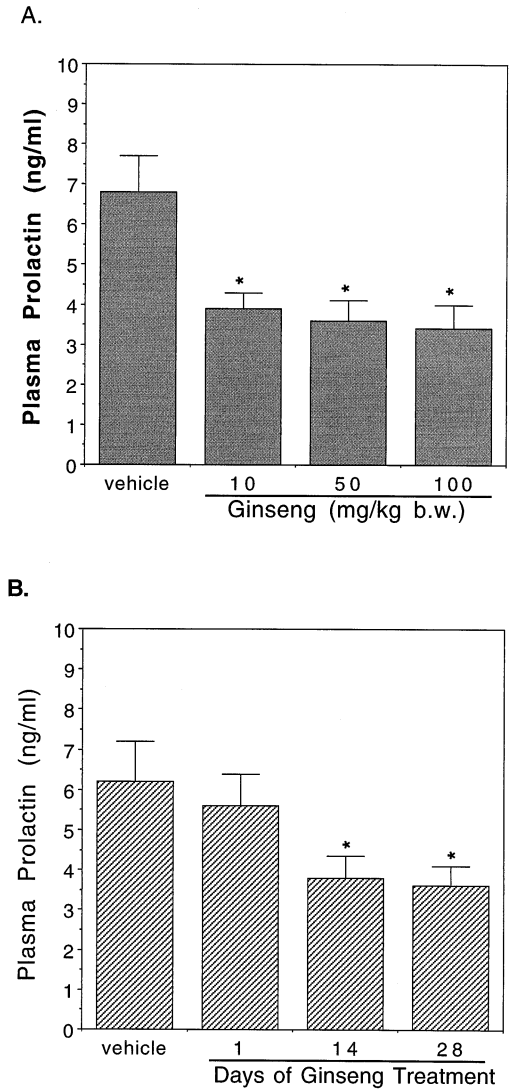

FIG. 3. Effect of ginseng doses (A) and duration of ginseng treatment (B) on plasma prolactin levels in male rats. Results are expressed as mean ± SEM with twelve animals per treatment group. Asterisks denote significance at $p < 0.05$ when compared to respective vehicle group.

Particularly striking was the significant decrease in prolactin levels that occurred in response to all ginseng doses tested. Because prolactin release is controlled primarily by inhibitory dopaminergic inputs to the anterior pituitary (16), this finding suggests that ginseng treatment may either influence central nervous system dopaminergic activity or affect prolactin by a direct pituitary action. There is considerable evidence that ginseng or its ginsenoside constituents affect central nervous system function (1,3,4,13,28,30). Indeed, behavioral studies have demonstrated that *P. ginseng* and specific ginsenosides increase spontaneous motor activity (28), inhibit food intake (30), affect learning and memory (1,22), and have anxiolytic actions (4,5,31). Moreover, *P. ginseng* and/or ginsenosides have been shown to enhance dopaminergic activity (28), and modulate GABAergic (13) and cholinergic (3) neurotransmission. Besides causing a decrease in plasma prolactin levels, increased dopaminergic activity (23), or pharmacological activation of dopamine receptors (11,24) has been shown to stimulate male rat copulatory behavior. Taken together, these results suggest that the ability of *P. quinquefolium* to enhance male copulatory behavior in rats and reduce plasma prolactin levels, is due to increased dopaminergic activity in the central nervous system. It has been reported that dopamine mediates the ability of *P. ginseng* to increase spontaneous motor activity (28). However, in a recent study, daily *P. quinquefolium* administration did not appear to alter spontaneous activity in adult male rats (personal communication with Dr. J. Ferraro, 1997). Therefore, the copulatory behavior results in *P. quinquefolium*-treated rats in the current study are unlikely to be due to a change in general activity.

Interestingly, ginseng has been shown to have direct effects on penile tissue which could play a role in its copulatory performance-enhancing actions. It was recently reported that *P. ginseng* extract enhanced nitric oxide release from the endothelial cells of rabbit penile corpus cavernosum and induced relaxation of the corpus cavernosum (7). In the current study, acute treatment (1 day) with the 100 mg/kg dose of *P. quinquefolium* did not significantly affect mount or intromission latency or plasma prolactin levels, but did significantly reduce ejaculation latency. These results suggest that acute ginseng treatment may affect copulatory performance, independent of purported changes in dopaminergic activity, via a direct action on penile tissue.

Ginseng is an essential component of traditional Oriental medicine for the treatment of sexual dysfunction (10). Clearly, further work is necessary to determine the site(s) and mechanism(s) of action of *P. quinquefolium* in its ability to facilitate male copulatory behavior and to determine whether the present results obtained in rats, may apply to other species as well.

ACKNOWLEDGEMENTS

The authors thank Clint Conner, Shelby Best, and Kelly Higgins for technical support and the Howard Hughes Medical Institute for financial support. In addition, we gratefully acknowledge the NIADDK for supplying the materials used for the LH and prolactin radioimmunoassays.

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