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Preventing L-NAME inhibitory effects on rat sexual behavior with hydralazine, isradipine or captopril co-treatment

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Abstract

The effects of the chronic oral treatment with $N^{(G)}$-nitro-L-arginine methyl ester (L-NAME), separately or in combination with isradipine, captopril or hydralazine, on standard and temporal patterning sexual behavior of male rats were evaluated. L-Arginine and filtered water were used as control. L-NAME treatment decreased the copulatory rate and hit rate factors of sexual behavior. However, the initiation factor and temporal patterning were less modified by the drug. After 14 days of L-NAME treatment suspension the male rat sexual response was recovered. The three antihypertensive agents were able to reverse partially or totally the inhibitory effects of L-NAME, suggesting that the chronic oral treatment with L-NAME induces penile erection dysfunction by peripheral mechanisms. The present results suggest that chronic oral treatment with nitric oxide (NO) synthase inhibitor can be a relevant and powerful peripheral erectile dysfunction model to evaluate the effects of drugs on erectile function of male rats.

Keywords: Sex; L-NAME; Nitric oxide; Antihypertensive agents; Hydralazine; Isradipine; Captopril; Penile erection dysfunction; Sexual behavior temporal patterning

1. Introduction

Penile erection depends on an increased blood flow to the corpora cavernosa induced by extra activity of sacral parasympathetic–nitrergic innervations and decreased sympathetic activity. "In vitro" experiments have shown that nitric oxide (NO) plays a crucial role on the vasodilator mechanism causing penile erection (Azadzoi et al., 1992; Ignaro et al., 1990; Kim et al., 1993; Bush et al., 1982; Palmer et al., 1987; McCann et al., 1999; Saenz de Tejada, 2002). NO also plays a relevant central nervous system role on the control of sexual behavior by promoting dopamine release in the medial preoptic area (MPOA) (Lorrain et al., 1996; Garthwaite and Boulton, 1995; Hull et al., 1997), and activating the luteinizing hormone–releasing hormone (LHRH) release (McCann et al., 1999).

Acute inhibition of NO synthesis by intraperitoneal injections of $N^{(G)}$-nitro-L-arginine methyl ester (L-NAME), a potent NO synthase inhibitor, has been shown to induce significant reduction of intromissions and ejaculations in rats (Soares de Moura et al., 1994; Benelli et al., 1995; Bialy et al., 1996; Hull et al., 1997). The L-NAME inhibitory effects on sexual behavior are due to a decrease in penis blood flow, an increase in the adrenergic drive to the corpora cavernosa and a decrease in dopamine released in hypothalamic MPOA (McCann et al., 1999; Hull et al., 1997). Considering that patients with arterial hypertension might have sexual impairment and also, that some antihypertensive drugs may also induce erectile dysfunction, the present study investigated the effects of three antihypertensive drugs on the inhibitory L-NAME effect on male rat sexual response: hydralazine, an endothelium-independent vasodilator compound; isradipine, a calcium-blocking agent; and captopril, an inhibitor of kininase II.

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2. Material and methods

2.1. Animals

Adult male Wistar naive rats (300–350 g) from our own colony were used. Animals were maintained five per cage in temperature-controlled colony rooms (23° ± 1 °C) on a 12-h light/dark cycle, with lights off at 6 a.m. Food and water were available ad libitum and ambient temperature was kept at 23 ± 1 °C. The Ethical Committee of UERJ approved the experimental protocols.

2.2. Drugs and procedural details

L-NAME, L-arginine, hydralazine and captopril were purchased from Sigma, USA. Isradipine was a gift from Novartis Biociência. All drugs were dissolved in filtered water for the experiment and administered orally in the drinking water.

Females were brought into estrous via subcutaneous injections of 100 μg/kg estradiol benzoate dissolved in corn oil 72 and 48 h before and 500 μg/kg medroxiprogesterone acetate 5 h before testing. The females were tested with nonexperimental sexually vigorous male rats, immediately before the experiment. Proceptivity and receptivity of female rats were evaluated using the Ferraz scale (Ferraz et al., 2001). Only those females that achieved three or four grades were used in the experiment.

2.3. Behavioral testing

All rats were tested before the beginning and only those that showed at least two copulatory series in 30 min were included in the testing. Mating tests were performed during the period of darkness (1 to 5 p.m.), under a red light. After 10 min of adaptation period, in a rectangular wood observation cage with a transparent front side (60 × 60 × 80 cm), a stimulus female rat was introduced into the cage and the copulatory behavior test started. The following measurements were recorded or calculated: mount latency, the time from onset of the test to the first mount with or without penile insertion; intromission latency, the time from the introduction of the female to first intromission; ejaculatory latency, time form the first intromission to ejaculation; mount number, the number of the mounts without intromission prior to ejaculation; intromission number, the number of mounts with intromission before ejaculation; postejaculatory interval, time from ejaculation to the first intromission of the second copulatory series; intercopulatory interval, the average interval between successive intromissions (calculated as ejaculation latency divided by intromission number + 1); copulatory efficiency, a measure of intromission success (calculated as percentage of mounts in which the male gained vaginal insertion); copulatory rate, the percentage of rats that showed ejaculation per group. Tests were terminated if intromission latency exceeded 15 min or ejaculation latency exceeded 30 min. The present study was made in the blind way as described in literature (Ferraz et al., 2001), with two observers evaluating the sexual behavior of each male rat in experimental or control groups. In case of discrepancy on observation between two observers, with relationship to the observed pattern (mounts, intromissions, ejaculations, genital grooming, female rat pacing behavior) or the time (mount latency, onset of each mount bout, etc.), the data were not considered.

2.4. Temporal patterning analysis

Results of mount bout analysis were expressed as: mount bout number, the sequence of mounts (one or more), with or without intromission, uninterrupted by any behavior that is not oriented toward the female; the timeout, the interval from the end of one mount bout to the start of the next mount bout; the intermount bout interval, the time from the onset of one mount bout to the start of the next mount bout; the mount bout time, the average time of mount bout preceding the ejaculation; the genital grooming rate, calculated as the time dispensed in genital autogrooming divided by total time dispensed in a copulatory series; intromissions per mount bout, calculated as intromission number divided by mount bout number; and total mounts per mount bout, calculated as the sum of intromissions and mounts divided by the mount bout number.

2.5. Experimental protocols

2.5.1. Effects of chronic treatment with L-NAME or L-arginine on male rat sexual behavior

Thirty male rats were divided in three groups respectively treated with vehicle (filtered water), L-NAME (70 mg/kg/day) or L-arginine (70 mg/kg/day) during 28 days. Each rat was tested immediately before drug treatment (T_0); weekly after the beginning of treatment (T_7, T_{14}, T_{21} and T_{28}); and after interruption of treatment (T_{35} and T_{42}).

2.6. Effects of hydralazine, isradipine or captopril on the inhibition of sexual behavior induced by L-NAME

Sixty male rats were divided in six groups ($n = 10$), orally treated respectively with vehicle, L-NAME (70 mg/kg/day), L-NAME (70 mg/kg/day) plus isradipine (0.1 mg/kg/day), L-NAME (70 mg/kg/day) plus hydralazine (20 mg/kg/day) or L-NAME (70 mg/kg/day) plus captopril (60 mg/kg/day). Each rat was tested immediately before the pharmacological treatment (T_0) and at 28 days of treatment (T_{28}).

2.7. Statistics

The one-way analysis of variance (ANOVA) and the Student–Newman–Keuls test for further multifactorial com-

parison between groups were used for parametric data. Nonparametric data were analyzed by Kruskal–Wallis test, followed by the Mann–Whitney *U* test for comparison purposes between two groups. Chi-square test was used for copulatory rate analysis. The following comparisons were made in Protocol 1: comparisons among the three groups, Control × L-NAME-treated rats × L-Arginine-treated rats, on each test and comparisons within L-NAME-treated rats on successive tests, before, during and after interruption of chronic treatment; Protocol 2: comparisons between L-NAME-treated rats and rats co-treated with L-NAME and each of the three antihypertensive agents, and comparisons among control rats and all experimental groups.

3. Results

3.1. Protocol 1

L-NAME chronic administration was followed by a time-dependent decrease in percentage of rats that ejaculated (Table 1). Percentage of males that ejaculated decreased significantly after 28 days of chronic treatment with L-NAME ($\chi^2 = 17.518$, $P \leq .001$). Although all the animals achieved mounting, only three rats treated with L-NAME reached ejaculation within 30 min of testing. Fourteen days after the L-NAME withdrawal (T_{42}), the rats partially recovered the sexual response, since the percentage of L-NAME-treated rats that ejaculated did not show statistical difference to control group ($\chi^2 = 1.848$, $P > .05$).

The effects of chronic treatment with L-NAME or L-arginine on male rats sexual behavior are summarized in Fig. 1. L-Arginine did not change the sexual behavior pattern of rats in comparison with vehicle-treated rats. After 14 days, the L-NAME-treated rats showed an inhibition of sexual response by an increase in the intromission latency [$F(2,26) = 5.59$, $P = .018$], when compared with the control group, in the postejaculatory interval [$F(2,27) = 3.69$, $P = .038$], in the mount number (KW = 9.79, $P = .007$), and a decrease in the copulatory efficiency [$F(2,27) = 15.72$, $P = .001$]. After 21 days, the effects of L-NAME were even more pronounced since the L-NAME-treated rats showed an increase in the intromission latency [$F(2,27) = 5.88$, $P = .008$], in the postejaculatory interval [$F(2,26) = 4.606$, $P = .02$], in the mount number (KW = 11.816, $P = .003$), in the mount latency [$F(2,26) = 4.54$, $P = .02$], in the ejaculation latency [$F(2,26) = 4.784$, $P = .017$], in the intercopulatory interval [$F(2,26) = 16.228$, $P = .001$], and a decrease in the copulatory efficiency [$F(2,26) = 15.725$, $P = .001$]. After 28 days, L-NAME drastically reduced the number of animals displaying male sexual behavior (Table 1). Therefore, the data did not show statistical difference. Fourteen days after L-NAME withdrawal, the rats recovered almost normal sexual function, as seen in increased copulatory efficiency [$F(2,14) = 613.9$, $P = .001$], decreased mount latency [$F(2,18) = 8.157$, $P = .01$], decreased intromission latency [$F(2,14) = 14.293$, $P = .001$], decreased ejaculatory latency [$F(2,14) = 28.374$, $P = .001$], decreased postejaculatory interval [$F(2,14) = 26.387$, $P = .001$] and decreased intercopulatory interval [$F(2,14) = 34.813$, $P = .001$], compared to their performance on Day 21 of chronic treatment. However, the postejaculatory interval persisted longer compared to controls ($P \leq .05$).

The effects of L-NAME and L-arginine on temporal patterning sexual behavior of male rats are presented in Fig. 2. After 14 days, the treatment with L-NAME increased the mount bout number (KW = 6.596, $P = .037$). After 21 days, the L-NAME-treated rats exhibited a considerable increase in the mount bout number (KW = 6.878, $P = .032$), and a decrease in the intromission per mount bout [$F(2,23) = 7.97$, $P = .002$], and in the mount per mount bout [$F(3,30) = 4.516$, $P = .05$], in relation to control group. The temporal patterning sexual behavior of L-NAME-treated rats remains different to control 14 days after drug withdrawal, because the mount bout number (KW = 6.596, $P = .05$), and the intromissions per mount bout [$F(3,30) = 11.285$, $P = .01$], continued smaller than control group. The chronic treatment with 70 mg/kg of L-arginine did not change the male rat temporal patterning sexual behavior in all the tests, when compared with control group.

Table 1
Percentage of male rats that achieved mount, intromission and ejaculation during and after chronic oral treatment with L-NAME or L-arginine

Groups (treatment)	*T* (days)	*n*	Rats mounting (%)	Rats intromitting (%)	Rats ejaculating (%)
Vehicle	0–42	10	100	100	100
L-Arginine	0–35	10	100	100	100
L-NAME	0–14	10	100	100	100
L-NAME	21	9	100	88.89	88.89
L-NAME	28	9	100	55.55	33.33*
L-NAME	35	9	88.89	44.44	33.33
L-NAME	42	9	100	66.67	66.67**

* $P \leq .001$ compared to L-NAME-treated and vehicle-treated rats.

** $P \leq .001$ compared to L-NAME-treated rats, copulatory rate at T_{28} and after the suspension of L-NAME treatment.

3.2. Protocol 2

3.2.1. L-NAME

As in Protocol 1, chronic treatment with L-NAME drastically inhibited the sexual behavior after 28 days of treatment, decreasing the percentage of rats that ejaculated ($\chi^2 = 14.679$, $P = .01$, Table 2). L-NAME also increased the mount latency [$F(5,53) = 27.844$, $P = .0001$], the intromission latency [$F(5,54) = 36.473$, $P = .0001$], the mount number (KW = 30.45, $P = .0001$), the ejaculation latency [$F(5,53) = 25.225$, $P = .001$], the postejaculatory interval [$F(5,53) = 12.122$, $P = .011$], the intercopulatory interval [$F(5,53) = 13.756$, $P = .0001$], and decreased the intromission number (KW = 19.816, $P = .0004$), and the copulatory efficiency [$F(5,53) = 10.047$, $P = .0001$] (Fig. 3). These data are in agreement with Protocol 1.

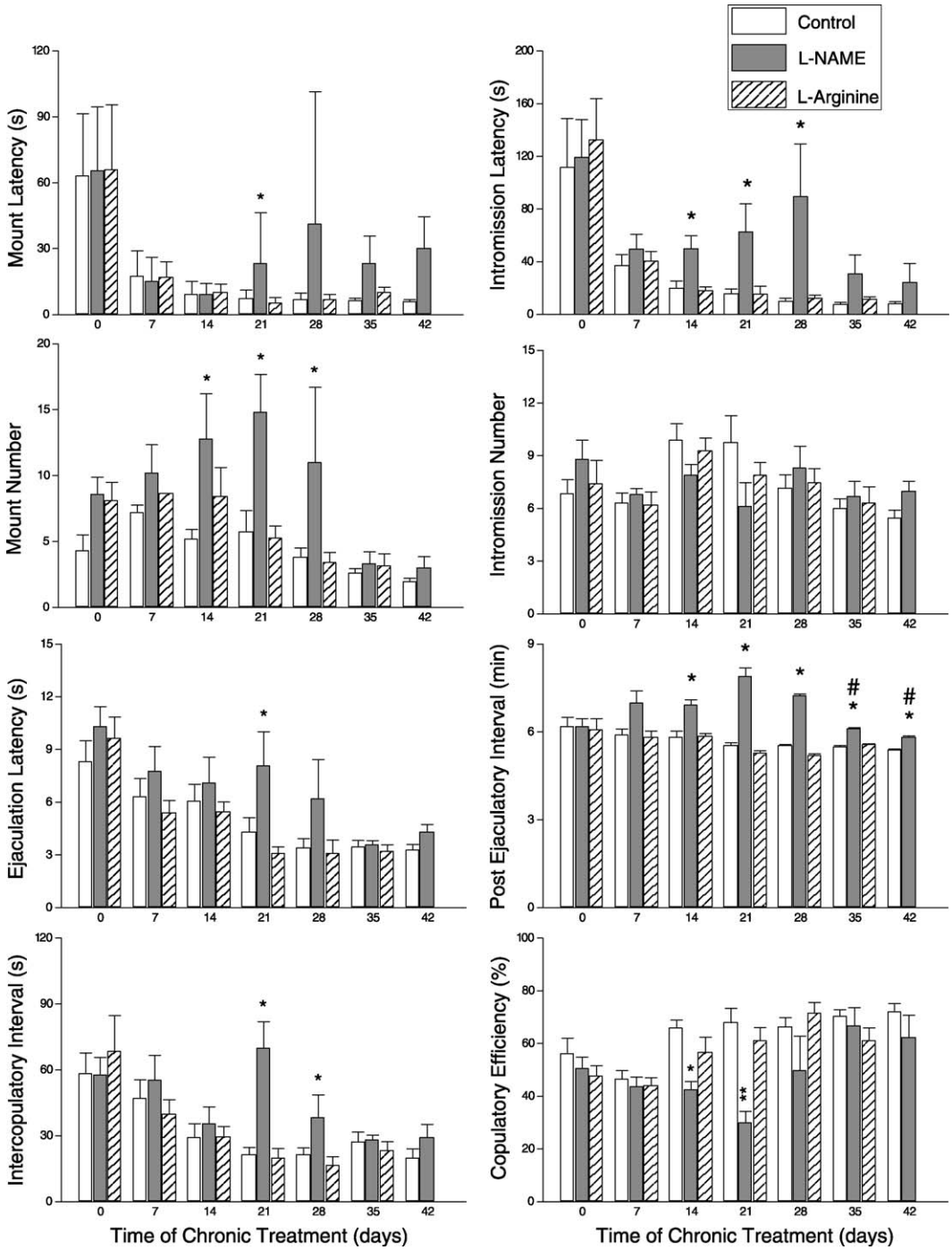

Fig. 1. Effects of chronic treatment with L-NAME (70 mg/kg/day) or L-arginine (70 mg/kg/day) on sexual behavior of male rats. Data are expressed as mean ± S.E.M. Statistical analysis made by one-way ANOVA or Kruskal–Wallis test. * $P \leq .05$, ** $P \leq .01$ for comparisons among control and experimental groups; $^{\#}P \leq .05$ for comparisons between L-NAME treated rats (T_{21}) and these groups after drug suspension (T_{35} and T_{42}).

3.2.2. Isradipine

The inhibitory effects of L-NAME on percentage of male that ejaculated were prevented by co-administration with the calcium channel blocker isradipine ($\chi^2 = 8.136$, P=.01, Table 2). Co-treatment with isradipine partially prevented the inhibitory effects of L-NAME on arousal measurements of sexual behavior, since isradipine reduced the mount latency [$F(2,26) = 26.54$, P=.001], and the intromission latency [$F(2,26) = 41.913$, P=.045], in comparison to L-NAME-treated rats, but never reached the control group response ($P < .05$). The effects of L-NAME in the intercopulatory interval [$F(2,26) = 15.404$, P=.005], and the cop-

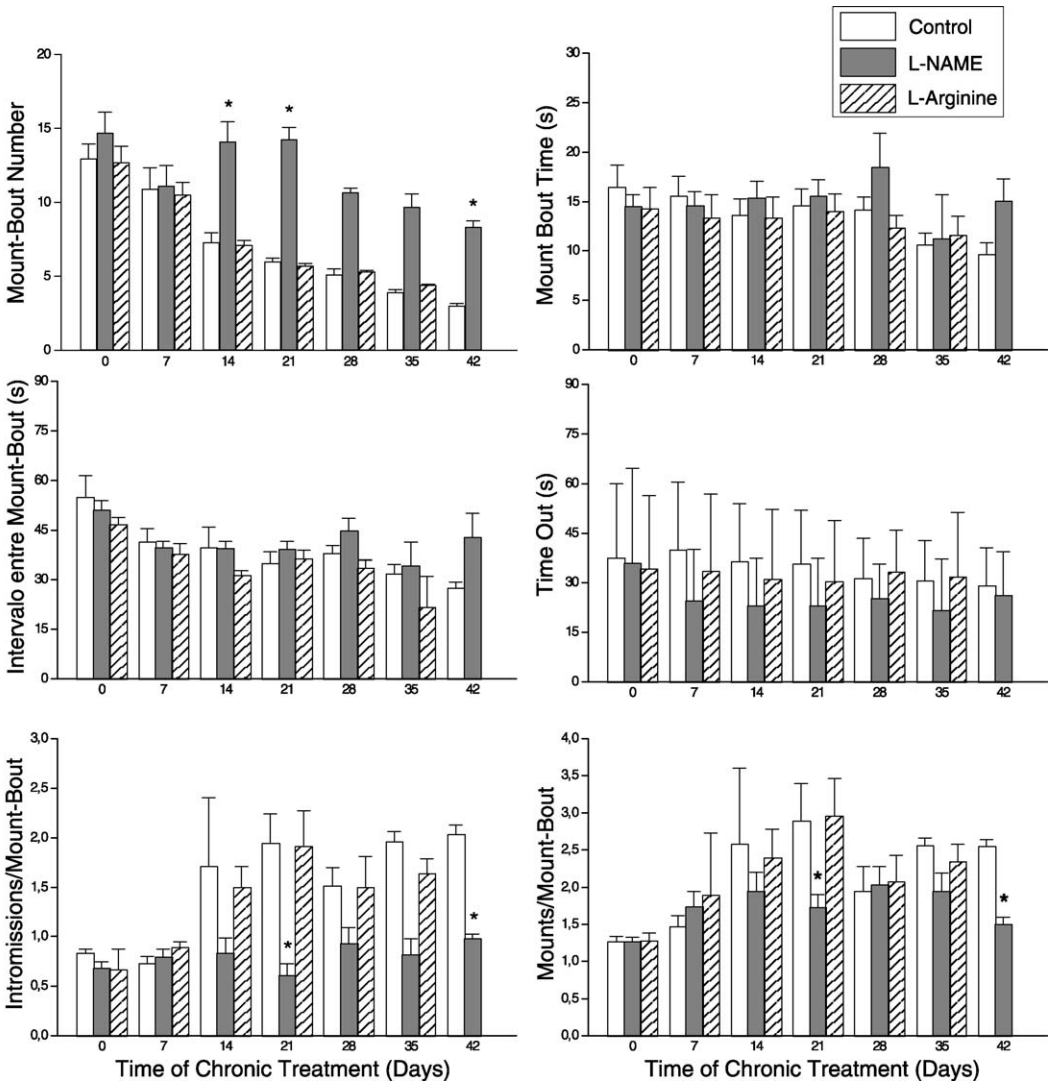

Fig. 2. Effects of chronic treatment with L-NAME (70 mg/kg/day) or L-arginine (70 mg/kg/day) on temporal patterning of sexual behavior of male rats. Data are expressed as mean ± S.E.M. Statistical analysis made by one-way ANOVA or Kruskal–Wallis test. * $P \leq .05$ for comparisons among control and experimental groups; # $P \leq .05$ for comparisons between L-NAME-treated rats (T_{21}) and these groups after drug suspension (T_{35} and T_{42}).

ulatory efficiency [$F(2,26) = 14.959$, $P = .008$], also were significantly inhibited by co-treatment with isradipine (Fig. 3), but also never reached the control group response.

Table 2
Percentage of male rats that achieved mount, intromission and ejaculation during and after chronic oral co-treatment with L-NAME and isradipine, hydralazine or captopril

Groups (treatment)	*n*	Rats mounting (%)	Rats intromitting (%)	Rats ejaculating (%)
Vehicle	10	100	100	100
L-Arginine	10	100	100	100
L-NAME	9	55.55	22.22*	22.22*
L-NAME + isradipine	9	100	70**	70**
L-NAME + hydralazine	9	100	60**	60**
L-NAME + captopril	9	100	70**	70**

* $P \leq .001$ compared to vehicle-treated rats.
** $P \leq .02$ compared to L-NAME-treated rats.

3.2.3. Captopril

The co-treatment with the kininase II inhibitor captopril prevents the inhibitory effects of L-NAME in percentage of male that ejaculated ($\chi^2 = 5.41$, $P = .02$, Table 2). Fig. 3 shows that the increase in intromission latency [$F(2,26) = 39.306$, $P = .0006$], and the decrease in intromission number (KW = 14.551, $P = .036$), and in copulatory efficiency [$F(2,26) = 14.959$, $P = .035$], induced by L-NAME were partially prevented by co-treatment with captopril. Finally, captopril completely prevented the increase in mount latency [$F(2,26) = 32.33$, $P = .0001$], and intercopulatory interval [$F(2,26) = 25.448$, $P = .0008$], induced by L-NAME (Fig. 3).

3.2.4. Hydralazine

The co-treatment with vasodilator hydralazine also prevented the inhibition of sexual behavior evoked by

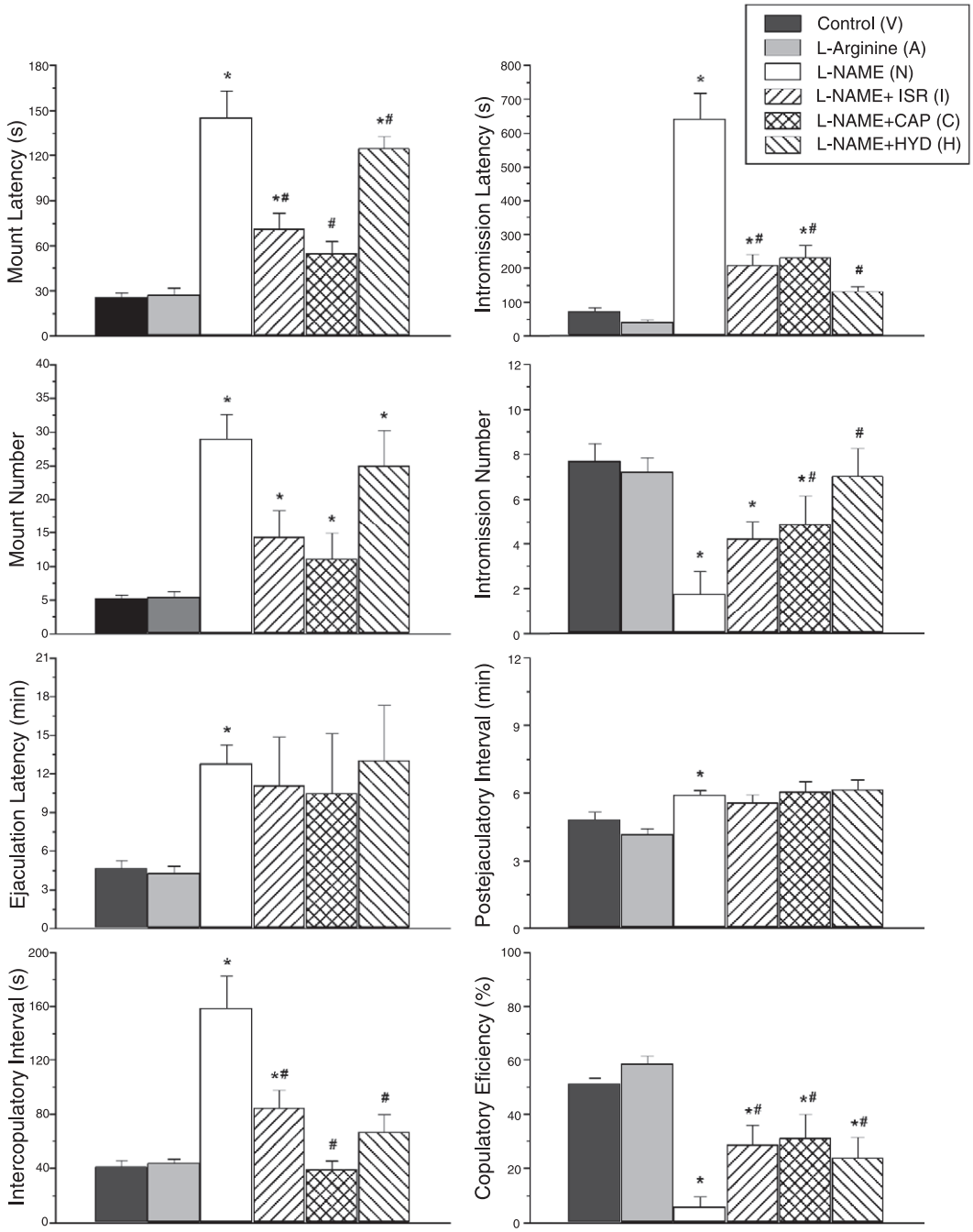

Fig. 3. Effects of captopril, isradipine or hydralazine on the inhibitory effect of L-NAME on the male rat sexual behavior. Data are expressed as mean ± S.E.M. Statistical analysis made by one-way ANOVA or Kruskal–Wallis test. * $P \leq .05$ for comparisons among control and experimental groups; $^{\#}P \leq .05$ for comparisons among L-NAME-treated rats (T_{21}) and L-NAME plus antihypertensive drugs treated rats.

L-NAME on percentage of male that ejaculated ($\chi^2 = 7.09$, $P=.01$, Table 2). Fig. 3 shows that hydralazine completely prevented the increase in the intromission latency [$F(2,26) = 35.041$, $P=.001$], and intercopulatory interval [$F(6,26) = 20.556$, $P=.0008$], and the decrease in intromission number (KW = 15.366, $P=.051$), induced by L-NAME. Hydralazine prevented the effects of L-NAME in the mount latency [$F(2,26) = 35.041$, $P=.001$], and copulatory efficiency [$F(6,26) = 20.556$, $P=.0008$], however, they never attained the control group response.

4. Discussion

The present results show that chronic treatment with NO synthase inhibitor L-NAME effectively decreased the male rat sexual response, confirming a preliminary report (Soares

de Moura et al., 1994), suggesting that these effects probably affect penile erection vascular mechanisms. However, the three different antihypertensives were able to prevent, at least partially, these L-NAME effects. The L-NAME inhibitory action, administered orally, on copulatory behavior became effective after 14 days of chronic treatment, increasing considerably after 21 days and almost completely inhibiting sexual response at 28 days, because L-NAME drastically reduced the number of rats that achieved intromissions and ejaculations. However, these effects caused no apparent irreversible vascular lesions, because the nearly normal sexual response was observed 2 weeks after the suspension of L-NAME treatment.

Several studies suggest that sexual behavior is produced by interaction among at least four distinct mechanisms, probably involving different neural structures, initially named by Sachs and Barfield (1970) as initiation factor (arousal component), intromission count factor, hit rate factor and copulatory rate factor, respectively. Contributing to the initiation factor are the mount and intromission latencies; contributing to the intromission count factor is the intromission number; contributing to the hit rate factor is the copulatory eficiency; and contributing to the copulatory rate factor are ejaculation latency, postejaculatory interval and intercopulatory interval (Sachs and Barfield, 1970; Sachs, 1978; Ferraz et al., 2001). Further studies have agreed that the initial two related factor parameters, mount and intromission latencies, allow the evaluation of the sexual response arousal component. The intromission number and the copulatory efficiency allow evaluating the erectile response. The ejaculatory latency and intercopulatory interval allow the evaluation of the ejaculatory component of sexual behavior (Ferraz et al., 2001). The temporal patterning of sexual behavior analysis probes the most complex socio-sexual behavior, and it also contributes understanding the arousal and sexual performance (Pfaus and Phillips, 1991; Yells et al., 1995; Ferraz et al., 2001). Therefore, each component of the male rat sexual response can be evaluated independently from the others. At 14 days of treatment, the L-NAME-treated rats appear to show difficulty in penile insertion, because the intromission latency, postejaculatory interval and mount number were effectively increased and the copulatory efficiency was reduced. On the other hand, the arousal mechanism was not altered, because the mount latency was not significantly modified, confirming a similar report on the lack of effect of acute injections of L-NAME on sexual arousal, coupled with impairment of copulatory efficiency (Hull et al., 1994). These data suggest that the copulatory rate and hit rate factors are the most sensitive component of sexual behavior with NO synthesis inhibition, and they are probably due to a decrease on NO-dependent vasodilator mechanisms and an increase on genital sympathetic transmission (Saenz de Tejada, 2002). However, we cannot discard the possibility that a central nervous system mechanism may be involved in the L-NAME-induced inhibitory effects on arousal component of male rat sexual behavior. These inhibitory effects of L-NAME were reverted by drug suspension, suggesting the absence of irreversible organic lesions after 28 days treatment. The ease of arousal and consummatory measures of male rat sexual response during successive copulation in controls and L-arginine-treated rats was due to a facilitatory effect of experience. It did not happen in L-NAME-treated rats, confirming the inhibitory effects of L-NAME on male rat sexual behavior.

The modifications on sexual behavior temporal patterning of male rats by chronic oral L-NAME treatment observed were an increase in the mount bout number and a reduction in the number of intromissions per mount bout and in the total number of mounts per mount bout. However, because the drug did not modify the onset time of each mount bout, neither did it change the social–sexual behavior of the animals, we suggest that these effects are not related to central pathways regulating temporal patterning sexual behavior, and it probably could be related to a peripheral erectile response dysfunction.

The three different antihypertensive agents effectively prevented the inhibitory action on copulatory behavior of L-NAME, because all drugs were effective increasing the percentage of males that ejaculated at the end of treatment. The inhibitory effects of L-NAME on parameters related to sexual behavior arousal mechanism were also partially prevented by the co-treatment with antihypertensive compounds. Captopril was the most effective in preventing an increase in the mount latency, while hydralazine was the most effective in preventing the L-NAME effect in the intromission latency. As the antihypertensive drug had no effect on the central nervous system, we can postulate that the effect of L-NAME on arousal factor is due to a peripheral mechanism. The inhibitory effects on erectile mechanism evoked by L-NAME were prevented by the three antihypertensive drugs, since those substances, at least partially, prevented the effect of L-NAME on the copulatory efficiency, and intercopulatory interval. In addition, captopril and hydralazine also prevented the L-NAME effect on the intromission number, suggesting that those drugs protect against L-NAME-induced penile erection impairment. However, despite the significant effect of the antihypertensive drug on the L-NAME inhibitory effect on erectile mechanism, co-treatment did not attain the control sexual response. The co-treatment did not prevent the inhibitory effects of L-NAME on ejaculatory latency, suggesting that ejaculatory inhibition induced by L-NAME might not be due to vasoconstriction. However, we cannot discard a hypothesis of a central mechanism being implicated in this phenomenon. Although all the sexual behavior components were inhibited by chronic treatment with L-NAME, these effects were prevented, at least partially, by co-treatment with the three different antihypertensive drugs. The effectiveness of the three antihypertensive agents in preventing either totally or partially the sexual improvement elicited by orally administered L-NAME suggests that these inhibitory effects probably are related to peripheral mechanisms delaying the penile erections.

5. Conclusions

The main findings are summarized as the follows: (a) the chronic treatment with NO synthase inhibitor L-NAME effectively decreased the sexual response of male rat; (b) the inhibitory effect of L-NAME on copulatory behavior is effective after 14 days of chronic treatment, increasing considerably at 28 days; (c) the main effect of chronic oral L-NAME treatment occurs in erectile function, because the hit rate and intromission count factors were more inhibited, while the arousal component was less altered; (d) the effects of L-NAME were, at least partially, reverted by suspension of drug, suggesting the absence of irreversible organic lesions; (e) the co-treatment with three different antihypertensive agents effectively prevents the inhibitory action induced by NO synthesis inhibition, probably due to the vasodilator effect of those compounds. However, we cannot discard a hypothesis of other mechanisms being implicated in this phenomenon. Finally, we suggest that the chronic oral treatment with L-NAME provides a relevant model of penile erection dysfunction peripherally mediated and, therefore, it is a powerful tool to evaluate the effects of new agents affecting the sexual function.

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