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Effect of Yohimbine on Nociceptive Threshold in Normoglycemic and Streptozotocin-Treated Hyperglycemic Mice[1]

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BANSINATH, M., K. RAMABADRAN, H. TURNDORF AND M. M. PUIG. *Effect of yohimbine on nociceptive threshold in normoglycemic and streptozotocin-treated hyperglycemic mice*. PHARMACOL BIOCHEM BEHAV 33(2) 459–463, 1989.—The effects of yohimbine (0.1, 1, 3 and 10 mg/kg SC) on nociceptive threshold were tested in mice using the tail-immersion and hot-plate tests. The tail-flick (withdrawal) latency, a monosynaptic spinal nociceptive response, was significantly lowered by yohimbine. This hyperalgesic response was at its peak 0.5 hr after yohimbine injection. The tail-flick latencies expressed as % of basal latencies were, 95 ± 8, 100 ± 10, 62 ± 10, 33 ± 7 and 28 ± 4 in vehicle and 0.1, 1, 3 and 10 mg/kg in yohimbine-treated groups respectively. Yohimbine-induced hyperalgesia was observed when stimulus temperature was either 50°C or 45°C; however, the opiate antagonist naloxone (3 mg/kg SC) induced a hyperalgesic response at 50°C and an analgesic response at 45°C stimulus temperature. Streptozotocin-induced hyperglycemia did not influence the hyperalgesic response of yohimbine. In the hot-plate (60°C) test, which involves higher centers and a polysynaptic nociceptive reflex, yohimbine did not modify the jump latency. The data provide evidence for the presence of a tonic spinal noradrenergic inhibitory control of nociceptive mechanism(s) which does not appear to be readily altered by hyperglycemia.

Yohimbine Nociception Tail flick Jump latency Normoglycemia Streptozotocin Hyperglycemia Hot-plate test Tail withdrawal Tail-immersion test

NOREPINEPHRINE is among the endogenous monoamines implicated in the regulation of the nociception. In fact, a role for norepinephrine has been considered in both narcotic- (11, 13, 24) and nonnarcotic-induced analgesia (19, 30, 33, 39). Both pre- and postsynaptic adrenergic alpha$_1$ and alpha$_2$ receptors have been implicated in modulating nociceptive responses (22, 32, 34, 38). Yohimbine, an adrenergic alpha$_2$ antagonist, has been used as a pharmacological probe to assess the involvement of adrenergic alpha$_2$ receptors in mediating antinociception (5, 12, 21). Some results indicate that yohimbine per se affects basal nociceptive responses. For instance, a biphasic dose-dependent effect of yohimbine was observed in the vocalization test with low doses (0.32–2 mg/kg) producing hyperalgesia and high doses (8–25 mg/kg) producing analgesia (23). Vocalization response, like jump response in the hot-plate test, is a multisynaptic reflex involving higher centers (25). However, the tail-flick response in rodent tail-immersion test is a spinal monosynaptic reflex (27). The conclusions on the extent of participation of spinal noradrenergic systems in the antinociceptive effects induced by central stimulation (5), morphine (18) or the adrenergic alpha$_2$ agonist, azepexole (38), administration have been shown to be dependent on the analgesic test used.

Hyperglycemia has been shown to modulate opiate pharmacodynamics (3, 4, 6). Furthermore, streptozotocin-induced hyperglycemia attenuates naloxone-induced hyperalgesia in the tail-immersion as well as in the hot-plate test, suggesting that the endogenous opioid system(s) is(are) altered by hyperglycemia (28). However, the effect of hyperglycemia on the noradrenergic component of pain responses has not yet been reported.

In the present study, the effect of yohimbine on basal nociceptive threshold was assessed in tail-immersion and hot-plate tests in normoglycemic and streptozotocin-induced diabetic mice. The results indicate that yohimbine lowers the nociceptive threshold in the tail-immersion test when the stimulus is either 45°C or 50°C. Streptozotocin-induced hyperglycemia does not alter yohimbine-induced hyperalgesia. Furthermore, in the hot-plate test, the hyperalgesic response of yohimbine cannot be demonstrated. These results provide evidence for the existence of tonically active

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spinal inhibitory noradrenergic pain pathways.

METHOD

Animals

Male Swiss-Webster mice weighing 25–30 g (Taconic Farms, PA) were housed five per cage in a room with controlled temperature (22 ± 2°C), humidity and artificial light (0630–1900 hr). The animals had free access to food and water and were used after a minimum of four days acclimation to the housing conditions.

Hyperglycemic Mice

Chronic hyperglycemia (diabetes) was induced by an intraperitoneal (IP) injection of 200 mg/kg streptozotocin (Sigma Chemical Co., St. Louis, MO) dissolved in 0.01 M citrate buffer at a pH of 4.0–4.5 and administered 7–8 days before the experimental day (3). Control animals received vehicle alone. The induction of diabetes was confirmed by measurement of blood glucose levels with Ames Dextrostix and a reflectance colorimeter (Accu-chek II, Boehringer Mannheim Diagnostics, Indianapolis, IN). Blood samples were collected from the retro-orbital sinus to measure blood glucose levels. On the day of the experiment, streptozotocin-treated animals had blood sugar levels of 450 ± 15 mg/dl, while citrate buffer-treated mice had sugar levels of 170 ± 13 mg/dl.

Drugs

Yohimbine hydrochloride and naloxone hydrochloride (Sigma Chemical Co., St. Louis, MO) were dissolved in distilled water just before use and were injected subcutaneously (SC) in a volume of 10 ml/kg. The doses of drugs refer to the salt form used. Animals in the control group received vehicle injections. A minimum of 8 animals were used per group.

Tail-Immersion Test

The details of the tail-immersion test procedure used were essentially similar to those published earlier (27, 37, 38). Briefly, using a circulating immersion water heater (Catalog No. 13-874-170, Fisher Scientific, Pittsburgh, PA), a constant temperature of 50 ± 0.2°C was maintained in the water bath in which the terminal 3 cm of the animal's tail was immersed. The nociceptive end point was characterized by a violent jerk of the tail (tail flick/withdrawal). During nociceptive reaction measurements, the animals were briefly immobilized (25–30 sec) by gentle wrapping in chux (Durasorb Utility Pad diapers, Professional Medical Products, Greenwood, SC). Previous results from our laboratory indicate that in the tail-immersion test, naloxone-induced hyperalgesia can be demonstrated in chux- but not in tube-restrained mice (26,27). Baseline latencies were determined twice, 5 min apart, and averaged to give a single predrug latency. Postdrug latencies were measured at 0.5, 1, 2 and 4 hours after yohimbine injection. In order to minimize tissue injury due to repeated exposure of the heat stimulus, a cut-off time of 15 sec was imposed.

The cross-over design. To obviate the anxiogenic effect of yohimbine (10) interfering with test results, a cross-over design was used. In some groups, yohimbine response was assessed in drug- and test-naive animals, while other groups of mice were familiarized to test procedure after vehicle treatment for two consecutive days (drug- but not test-naive mice) and on third day the effect of yohimbine was assessed. The drug- and test-naive groups were allowed a washout period of two days after yohimbine injection and the tail-flick test was repeated in these mice using the vehicle as treatment.

The stimulus temperature as a factor affecting yohimbine response. In a separate set of experiments, the effect of yohimbine was assessed in the tail-immersion test when stimulus temperature was kept at 45°C. In naloxone-treated groups, postdrug latencies were measured 0.25 hours after injection (26–28).

Hot-Plate Test

The hot plate analgesia meter (model 35 D, IITC, Landing, NJ) was used with surface temperature maintained at 60 ± 0.5°C. Accuracy of the temperature setting was monitored and verified using a surface thermometer. After placing the mouse on the hot plate, the reaction time for escape attempt (jump) was recorded in seconds (28). In order to avoid the effect of learning (14, 20, 29), latency times were determined in separate groups of mice, 30 min after vehicle or yohimbine (0.1, 1, 3 and 10 mg/kg) treatment. A cut-off time of 60 sec was used.

Data Analysis

In the tail-immersion test, postdrug latencies were calculated as percentage of the respective animal's control latency. For each animal, the area under curve (AUC) between % of basal latency and each time point over a 4-hour period after yohimbine treatment was determined by the linear trapezoidal rule (35). For a given dose, the determination AUC integrates both the intensity as well as duration of response (3). In the hot-plate test, jump latency in yohimbine-treated mice was expressed as percentage of mean latency of the vehicle-treated group.

The data are presented as group means (± SEM). From the data of the tail-immersion test, an index of nociceptive threshold was calculated using the formula: (mean AUC in yohimbine-treated group/mean AUC in vehicle-treated group) × 100. This index of the nociceptive threshold was subjected to linear regression analysis in order to calculate ED_{50} and confidence limits. The statistical significance of the data was assessed by ANOVA followed by Newman-Keuls test using a computer program (9). A p value of <0.05 was considered significant.

RESULTS

Tail-Immersion Test

Effect of yohimbine on tail-withdrawal latency in drug- and test-naive mice. Tail-withdrawal latencies at the stimulus temperature of 50°C, expressed as % of basal latencies after vehicle or yohimbine (3 mg/kg) in drug- and test-naive mice are presented in Fig. 1. Tail-withdrawal latencies in the vehicle-treated mice were not significantly affected by repeated testing. However, yohimbine (3 mg/kg) treatment significantly ($p<0.05$) decreased tail-flick latencies at all time points tested.

Effect of yohimbine on tail-withdrawal latency in drug- but not test-naive mice. When % of basal latency data from the vehicle, 0.1, 1, 3 and 10 mg/kg yohimbine-injected groups were subjected to the statistical analysis, ANOVA indicated that the dose of yohimbine had a significant effect, $F(4,111) = 20.59$, $p<0.0005$, and preexposure of mice to test conditions (drug- but not test-naive condition) did not interfere with the hyperalgesic response of yohimbine, $F(1,111) = 1.45$, $p = 0.229$, indicating that the anxiogenic effect of yohimbine does not influence the results.

The intensity and duration of hyperalgesic response of yohimbine. The AUC data from both drug- and test-naive, as well as drug- but not test-naive mice were pooled and subjected to

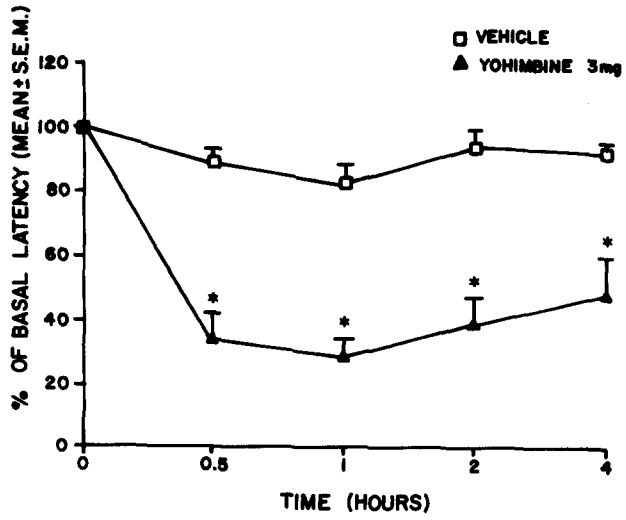

FIG. 1. Time-course of tail-withdrawal latency expressed as % of basal in vehicle- (square) and yohimbine- (3 mg/kg) (triangle) treated mice. *$p<0.05$. (Stimulus temperature = 50°C.)

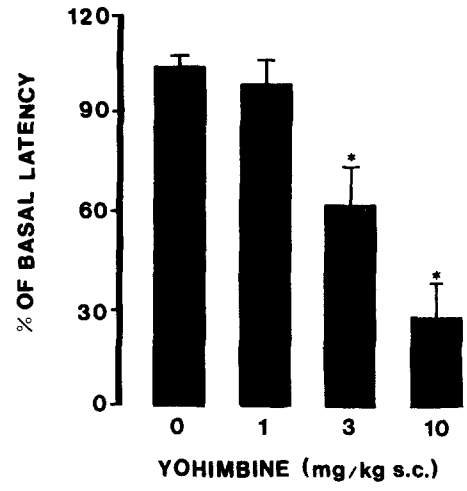

FIG. 3. Tail-withdrawal latency 30 min after the vehicle (0) or yohimbine treatment in tail-immersion test with the stimulus temperature of 45°C. *$p<0.05$.

ANOVA (Fig. 2). Yohimbine induced a dose-dependent hyperalgesic response, $F(4,104) = 37$, $p<0.0001$ (Fig. 2). The calculated ED_{50} (confidence limits) for yohimbine-induced hyperalgesia was 4.79 (2.2–10.3) mg/kg.

Effect of stimulus temperature on the hyperalgesic response to yohimbine and naloxone. Yohimbine produced a significant hyperalgesic response when stimulus temperature was 45°C, $F(3,32) = 10.86$, $p<0.0002$ (Fig. 3). A comparison of tail-flick latencies at 45 and 50°C stimulus temperature in vehicle-, naloxone- (3 mg/kg, 15 min postdrug) and yohimbine- (3 mg/kg, 30 min postdrug) treated mice is provided in Fig. 4. Vehicle treatment did not affect basal latency at either stimulus temperature. At 45°C, the opiate antagonist, naloxone, increased tail-flick latency, while it decreased tail-flick latency at 50°C.

Yohimbine-induced hyperalgesic response in hyperglycemic mice. The effect of yohimbine in citrate buffer-treated normoglycemic controls and in diabetic mice is represented in Table 1. In these groups, ANOVA indicated that yohimbine dose, $F(3,102) = 19.8$, $p<0.0005$, but not glycemic condition, $F(1,102) = 1.53$, $p = 0.22$, had a significant effect suggesting that yohimbine-induced decrease in tail-withdrawal latency is not affected by hyperglycemia.

Hot-Plate Test

The mean (± SEM) jump latencies in the hot-plate test were 34 ± 4, 28 ± 4, 27 ± 5 and 33 ± 5 in vehicle- and 1, 3 and 10 mg/kg of yohimbine- (SC) treated groups respectively. In diabetic mice, jump latencies (mean ± SEM) were 47 ± 6, 32 ± 6, 41 ± 7 and 44 ± 6 respectively, for vehicle, 1, 3 and 10 m/kg of yohimbine

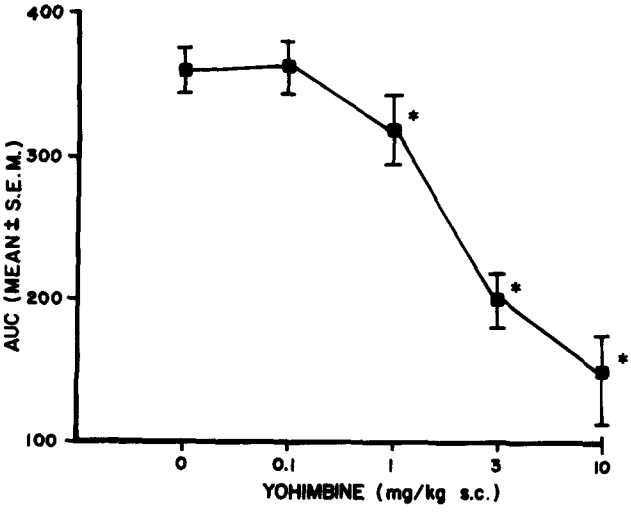

FIG. 2. Area under the curve (see the Method section) indicating the dose-effect relationship of the hyperalgesic response to yohimbine in the tail-immersion test. *$p<0.05$. (Stimulus temperature = 50°C.)

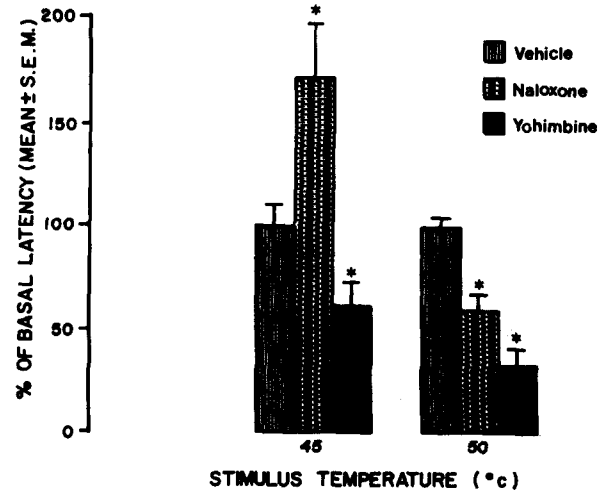

FIG. 4. Effects of vehicle, naloxone (3 mg/kg SC) and yohimbine (3 mg/kg) on tail-withdrawal latency when the stimulus temperature is 45 or 50°C. *$p<0.05$.

TABLE 1

THE HYPERALGESIC EFFECT OF YOHIMBINE IN NORMOGLYCEMIC AND HYPERGLYCEMIC (STREPTOZOTOCIN-INDUCED) MICE (TAIL IMMERSION TEST; 0.5 HR POSTDRUG DATA; STIMULUS TEMPERATURE = 50°C; n≥8 PER GROUP)

Dose* mg/kg	Tail Flick Latency as % of Basal (mean ± SE) Normoglycemic	Hyperglycemic
0	88.4 ± 5.5	90.9 ± 5.3
1	56.8 ± 7.6†	58.0 ± 10.6†
3	34.1 ± 6.7†	48.6 ± 8.7†
10	29.2 ± 3.2†	38.4 ± 5.0†

*ANOVA, $F(3,102) = 19.8$, $p<0.0005$. †$p<0.05$ when compared with the respective control (0 mg/kg) groups.

(SC) treatment. These results indicate that yohimbine does not modify jump latency either in normoglycemic or hyperglycemic conditions.

DISCUSSION

The present results indicate that the spinal nociceptive reaction is enhanced by yohimbine. Further studies using other adrenergic alpha$_1$ and alpha$_2$ antagonists will be helpful to test the hypothesis that spinal nociceptive mechanisms are tonically modulated by alpha$_2$ adrenergic receptors. However, alternative sites of action like the locus coeruleus autoreceptors and/or other descending system(s) originating from the brain stem may also be implicated in yohimbine-induced hyperalgesia.

The data from the tail-flick test in the present study are qualitatively comparable to that of the opiate antagonist, naloxone (26,27). However, unlike naloxone-induced hyperalgesia, yohimbine-induced hyperalgesia is not affected by stimulus temperature. Although naloxone-induced hyperalgesia is reported to be attenuated by hyperglycemia (28), yohimbine-induced hyperalgesia is not affected by hyperglycemia. Furthermore, yohimbine-induced hyperalgesia lasts longer (>4 hours) than naloxone-induced hyperalgesia (27).

Available literature on the effect of yohimbine on nociceptive threshold does not always indicate a constant effect. In the tail-flick test in rats, some studies report no change in basal latency after yohimbine (11, 16, 17, 21), while others indicate a hyperalgesic response either after intraperitoneal (7) or intrathecal administration (5, 15, 33). A recent report using intrathecal administration of yohimbine indicates no change in tail-flick latency (36). The present results confirm the hyperalgesic response to yohimbine in mice and extend the observation to systemic administration of yohimbine both in normoglycemic and hyperglycemic states. Methodological discrepancies as possible reasons for a similar disparity in the reported results on the hyperalgesic response to naloxone in tail-immersion test has been discussed elsewhere (27).

In the hot-plate test in rats, some studies indicate that yohimbine mimics opiates and induces analgesia (11,17). In addition, intraperitoneal or intrathecal administration of yohimbine has been shown to induce hyperalgesia (8,33). However, yohimbine (10 but not 1 mg/kg IP) has also been reported to increase reaction latency in the hot-plate test in mice (1,2). On the contrary, as observed in the present study, one report found no hyperalgesic response to yohimbine in mice (31). Repeated exposure of the same animal to the hot plate (1,2) varied dose and route of administration, and species used appear to be some of the possible reasons for the divergent results.

Taken together, the present results and the reports available in the literature after intrathecal yohimbine administration suggest that spinal nociceptive mechanisms are tonically inhibited by the adrenergic alpha$_2$ system. The present data also suggest a differential modulation of spinal noradrenergic and opioid nociceptive reflexes during hyperglycemic conditions. In addition, the descending tonic opioid and adrenergic nociceptive pathways appear to be differentially susceptible to the variations in stimulus temperature. Therefore, while considering the discrepancies in the literature, the need to consider methodological differences as underlying causes cannot be overemphasized.

Most of the studies which have reported data on yohimbine-induced changes in nociceptive threshold have used yohimbine as a pharmacological tool to test functional participation of adrenergic systems. Therefore, the time-course and dose-dependency of the effect of yohimbine on nociceptive threshold has not yet been reported. The present study has assessed the time-course and dose-response of yohimbine on nociceptive threshold in mice using two tests which involve different levels of integration of pain responses. The results suggest the existence of tonically-active spinal inhibitory noradrenergic pain pathways and indicate that hyperglycemia doses not affect the noradrenergic component of thermonociceptive reactions.

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