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Characteristics of Carbon Dioxide-Induced Antinociception

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MISCHLER, S. A., L. B. HOUGH AND A. H. BATTLES. *Characteristics of carbon dioxide-induced antinociception.* PHARMACOL BIOCHEM BEHAV 53(1) 205–212, 1996.—This lab previously showed that brief inhalation of high concentrations of CO_2 results in a prolonged, moderate antinociception with characteristics of a nonopiate, hormonal mechanism. To further characterize and optimize this response, the effect of a variety of methodological, biological, and stress-related manipulations were studied. No significant differences were found in the CO_2-induced response between animals that were tested during different portions of their diurnal cycles, in rats that were unhandled or habituated to nociceptive testing conditions, in male vs. female rats, or in animals of differing weights. Additionally, restraining animals prior to CO_2 exposure induced a hot plate antinociceptive response that was not different from the response produced by CO_2 alone. In contrast, on the tail flick test, a CO_2 -restraint interaction both increased and decreased the response at different times. The present findings show that CO_2 antinociception: a) is a reliable phenomenon not altered by a variety of methodological and biological conditions, and b) has characteristics of a novel, stress-mediated antinociceptive response.

CO_2 Antinociception Stress Restraint Brain Rat

THE PHENOMENON of environmental or stress-induced analgesia has gained widespread scientific acceptance, and has provided one framework in which endogenous pain-inhibitory systems have been studied. Following initial observations that acute exposure to stressful events such as foot shock leads to an increase in pain thresholds [(29,45), and see (7)], several laboratories confirmed that analgesia also resulted from environmental manipulations such as cold- or warm-water swims, food deprivation, immobilization, restraint, rotation, glucodeprivation, and others (8,9,29,60).

Recent work in this laboratory showed that inhalation of CO_2 (>70%) for 30 s resulted in short-term (1–2 min) anesthesia followed by prolonged (up to 60 min) mild antinociception (equivalent to that of 4 mg/kg morphine; IP), detectable with thermal and mechanical nociceptive tests (49). Following CO_2 exposure, animals regained locomotor control within 90 s, and appeared to completely recover from anesthesia within 5 min. Further characterization of this phenomenon showed that the antinociceptive response was not due to CO_2-induced hypoxia or prolonged acidosis, was not blocked by the opiate antagonist, naltrexone (0.1–10 mg/kg; IP), and was abolished in hypophysectomized animals (49). Taken together, these findings suggest that CO_2-induced antinociception represents a novel form of environmentally induced analgesia, mediated by a nonopiate, hormonal substance. The present CO_2 model of stress-induced analgesia exhibits unique characteristics not previously reported by investigators of other forms of stress-induced analgesia. CO_2 exposure has biological relevancy in anesthesia and euthanasia of laboratory animals.

There are many cases in which subtle variations in intensity or duration of stress exposure alter the nature of the stress-induced analgesic responses (44,55,59,60). Similarly, differential sensitivity of multiple forms of stress analgesia to opiate antagonists or to hormonal influences are related to biological differences (i.e., age and gender) in the test subjects (37,40, 41). Thus, it was our hypothesis that manipulation of methodological (lighting conditions and habituation to handling) as well as biological (gender and animal weight) factors would optimize the antinociceptive effect and reduce the variability of response to CO_2. In addition, because the antinociceptive effect induced by restraint and CO_2 are in some ways similar [see Amir and Amit (2); and Mischler et al. (49)], and because

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restraint is known to potentiate some kinds of antinociceptive responses (10), we hypothesized that restraint would modify the CO_2-induced response. Such a finding would further our understanding of the role of stress in the mechanism of action of CO_2 antinociception.

METHOD

Animals

Except where noted, male Sprague–Dawley rats (200 to 350 g; Taconic Farms, Germantown, NY) were used in these experiments. All animals were maintained in an AAALAC accredited facility. In gender experiments, weight-matched male and female rats were tested, and in weight-comparison studies, 200–600 g male rats were used. Animals were housed (two to three/cage) in polycarbonate rodent boxes, were maintained on a 12 L : 12 D cycle and tested between 2 and 8 h into the light cycle (except where noted below) and were given food and water ad lib. All procedures were reviewed and approved by the Institutional Animal Care and Use Committee of Albany Medical College.

Nociceptive Testing

Pain sensitivity was measured by hot plate and tail flick nociceptive tests. Hot plate nociception was measured by recording the latency to hindleg lift, stamp, or lick response after placing the subject on a 52°C surface (20); the most frequently observed response was the hindleg lift and lick. Upon responding, or after a maximum exposure of 60 s, the rat was removed from the heated surface. Animals exhibiting baseline values greater than 20 s or animals exhibiting persistent jumping on the hot plate test were discarded.

Tail flick nociception was determined by a modified (25) version of the radiant-heat tail flick test (17). Latency was recorded from the onset of the application of radiant heat until the animal moved its tail from the heat source. Baseline tail flick testing consisted of five tests at 1-min intervals; the last three tests were averaged and used as the baseline score. Baseline latencies were between 3 and 4.5 s. At subsequent time points, single measurements were made. A maximum exposure of 15 s was allowed.

Experimental Procedure

Approximately 8 min after baseline latencies were recorded, rats were exposed for 30 s to air (control; bench top compressed air source) or 100% CO_2 (purity = 99.5%; AWESCO, Albany, NY), by placing animals in a polypropylene chamber (22 × 35 × 22 cm), which was precharged (30 s) with the gas. A gas outlet near the top of the chamber prevented pressurization. Following exposure to the gas, animals were returned to their original cage. Nociception was measured at 10, 25, 40, and 60 min after initial exposure to each gas.

In some experiments, animals were habituated by daily handling for 3 days prior to the day of tail flick or hot plate nociceptive testing. Habituation for tail flick-tested animals consisted of removing the animal from its home cage, placing the animal under a loosely held towel, and manipulating its tail at 1-min intervals for 3 min, placing the animal in the uncharged, air-filled anesthetizing chamber for 60 s, and then returning the animal to its home cage. Habituation of hot plate-tested animals was similar to the tail flick habituation procedure except that following exposure to the anesthetizing chamber, rats were placed onto the unheated, hot plate surface for 60 s before being returned to their home cage. Nonhabituated control animals were not removed from housing quarters, handled, nor exposed to the nociceptive apparatus until the day of testing.

In other experiments, naive animals were acutely restrained (for approximately 8 min) by placing them in commercial Plexiglas restraint cylinders (7.5 cm i.d. × 20 cm; Harvard Apparatus, South Natick, MA) within 30 s following baseline testing. Rats were then exposed to CO_2 or air, and tested as previously described. Control (nonrestrained) animals were returned to their home cage following baseline testing.

Data Analysis

Antinociceptive scores were calculated as a percent of the maximum possible effect (%MPE) at each time point after gas exposure:

$$\%\text{MPE} = \frac{(\text{posttreatment latency} - \text{baseline latency})}{(\text{cut-off latency} - \text{baseline latency})} \times 100.$$

% MPE values were subjected to either one- or two-factor analysis of variance (ANOVA), and when appropriate, LSD post hoc comparisons were performed (CSS Statistica program, Tulsa, OK). The results are expressed as mean % MPE ± standard error of the mean (SEM).

RESULTS

Methodological Parameters: Optimizing CO_2-Induced Antinociception

In an attempt to optimize test parameters, CO_2-induced antinociception was studied in normal and reverse light-cycle animals, as well as in animals habituated to daily handling. Recovery from CO_2-induced anesthesia was followed by moderate hot plate antinociception in animals housed under normal lighting conditions, as well as in animals subjected to a reversed light:dark cycle (Fig. 1); ANOVA showed no differences in these experimental groups, $F(1, 92) = 1.16$, $p > 0.05$. Antinociceptive differences between air and CO_2-exposed animals were evident on the hot plate tests at 25 and 40 min following gas exposure in both experimental groups, $F(1, 92) = 16.26$, $p < 0.05$. Antinociception in animals exposed to normal lighting conditions returned to control levels by 60 min following gas exposure; antinociception in the reversed light-cycle group remained elevated ($p < 0.05$) at 60 min postexposure. Because no differences were found between light-cycle groups, in subsequent studies, animals were maintained under normal lighting regimens.

To determine if reduction of stressors associated with acute exposure of the animals to the testing paradigms and experimenter would change the CO_2-induced response, animals were habituated to the handling and testing procedures for 3 days prior to the day of hot plate nociceptive testing. Inhalation of CO_2 resulted in antinociception in both habituated and naive animals, $F(1, 41) = 18.91$, $p < 0.0001$ (Fig. 2), and there were no differences between naive and habituated animals $F(1, 41) = 0.29$, $p > 0.05$. CO_2-induced antinociception was seen at 10, 25, 40, and 60 min following gas exposure in naive animals ($p < 0.01$) and at 25 ($p < 0.01$) and 60 ($p < 0.05$) min following gas exposure in handled animals. Similar results were observed in animals habituated 2 and 4 days prior to the day of test (data not shown).

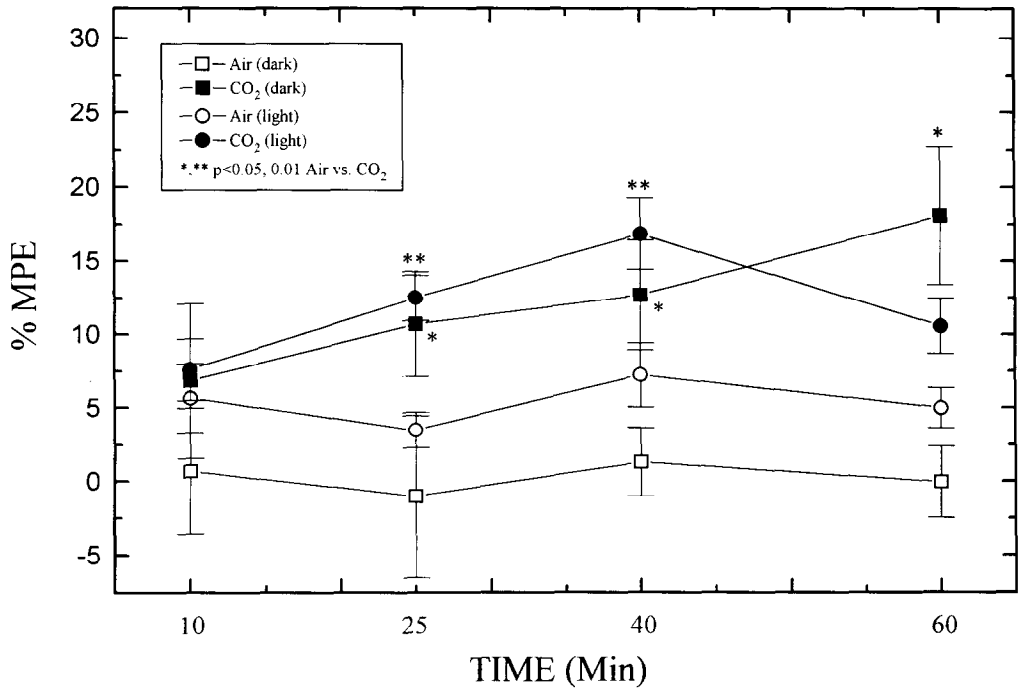


FIG. 1. CO_2-induced hot plate antinociception in normal and reversed light-cycled animals. Rats were housed in rooms maintained on a normal (lights on between 0700 and 1900; signified as light, circle; n = 31-47) or reversed (lights on between 1900 and 0700; signified as dark, square; n = 8-10) lighting cycle for at least 7 days prior to testing. All animals were tested between the hours of 0900 and 1500 in a normally lighted laboratory. Rats were tested for baseline antinociception, exposed (30 s) to either CO_2 (filled symbols) or air (open symbols), and were retested at the indicated times (min, abscissa). Baseline means were 10.63 ± 1.21 and 10.83 ± 1.24 (s, air vs. CO_2, respectively) in dark-cycled rats; 9.37 ± 0.39 and 9.71 ± 0.36 (s, air vs. CO_2, respectively) in light-cycled rats and did not differ between groups, Fs < 2.92, $p > 0.05$. Antinociceptive scores (%MPE, ordinate, mean ± SEM) are shown for each group. A two-factor (gas, light cycle) repeated measures (time) ANOVA showed a significant main effect of gas, $F(1, 92) = 16.26, p < 0.0005$, but failed to show a main effect of light cycle, $F(1, 92) = 1.16, p > 0.05$. No interactions were observed, Fs < 1.16. *,**$p < 0.05, 0.01$ for the difference between air vs. CO_2 in animals within the same diurnal group at the same time.

Evaluation of Biological Differences in CO_2-Induced Antinociception

CO_2-induced antinociception was also studied in male animals raised to different weights and in female animals. Inhalation of CO_2 resulted in a hot plate antinociceptive response at 25 and 40 min following gas exposure in both small (< 300 g, n = 22-44) and large (> 300 g, n = 25-27) rats, although no differences in the CO_2-induced hot plate responses were observed between the two groups, $F(1, 114) = 0.46, p > 0.05$ (data not shown). In similar experiments, male and female rats (both 200-350 g) showed no significant gender differences in CO_2-induced antinociception, $F(1, 32) = 0.75, p > 0.05$ (data not shown, n = 7-13).

Characterization of the Relationship Between CO_2-Induced Antinociception and Restraint

Because restraint can induce a stress analgesia and can modify opiate analgesia, the effect of restraint stress on CO_2-induced antinociception was studied. CO_2-induced anesthesia was followed by antinociception in both restrained and unrestrained rats tested by both the hot plate and tail flick methods (Fig. 3A,B). On the hot plate test, the antinociceptive response of restrained animals was significantly different from that of unrestrained control rats, $F(1, 38) = 4.65, p < 0.05$ (Fig. 3A); no restraint by group interaction was observed, Fs < 0.17, $p > 0.05$. Restraint-induced antinociception was present in both air and CO_2 groups at 10 min ($p < 0.005$) and in the air control group at 25 min ($p < 0.05$) following gas exposure. Furthermore, CO_2-treated animals showed significant antinociception, when compared to air control animals, 25 and 40 min following gas exposure in both unrestrained and restrained animals, $F(1, 38) = 16.19, p < 0.001$.

As described for the hot plate test, CO_2-treated animals showed significant antinociception compared to air controls on the tail flick test in both restrained and unrestrained animals, $F(1, 44) = 7.74, p < 0.01$ (Fig. 3B). In contrast to results achieved with the hot plate test, however, interactions between gas exposure and restraint groups in tail flick latencies were observed $F(3, 132) = 4.75, p < 0.005$. In nonrestrained animals, CO_2-induced antinociception was observed at 25 and 40 min after gas exposure ($p < 0.05$). In contrast, with restrained animals, CO_2 produced no effect at 25 min, but induced antinociception at 40 and 60 min ($p < 0.01$), and abolished the antinociception (relative to air controls; $p < 0.05$) at 10 min following exposure to the gas. In addition, restraint induced an antinociceptive response 10 min after exposure to air alone ($p < 0.01$); and potentiated the CO_2-induced response observed at 40 min following gas exposure ($p < 0.01$).

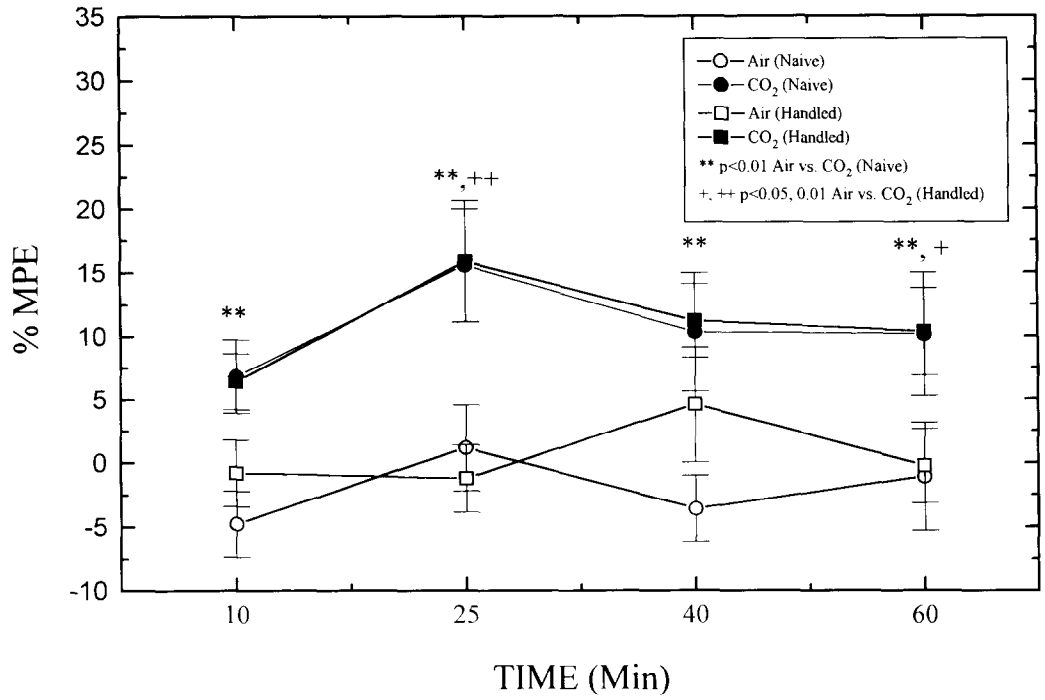
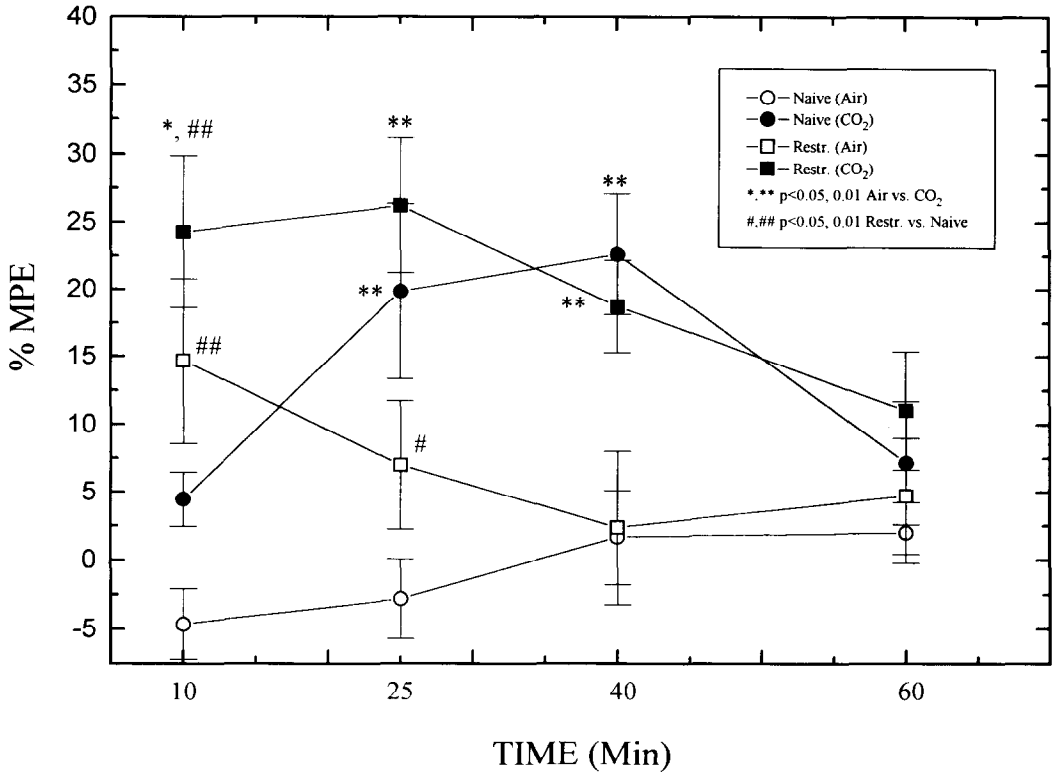


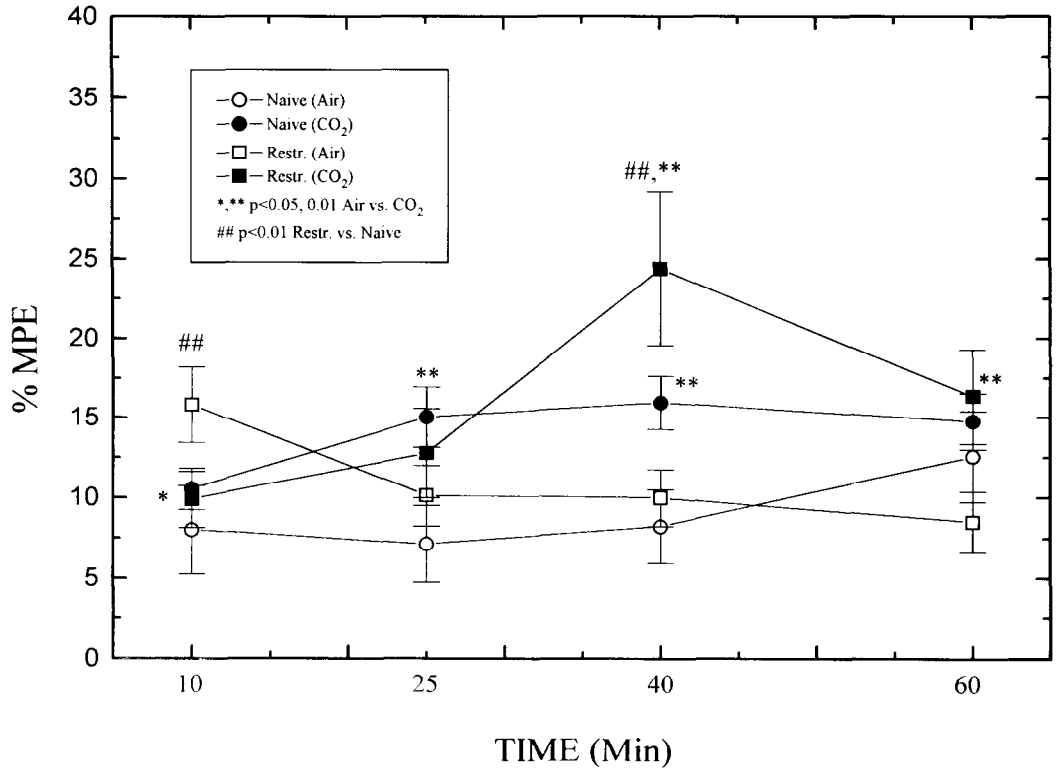
FIG. 2. CO_2-induced hot plate antinociception in naive animals and animals habituated to daily handling. Animals were habituated for 3 days as described, prior to gas exposure. On the test day, habituated (squares, n = 10–12) or naive, unhandled control rats (circles, n = 11–12) were tested for baseline antinociception, were exposed (30 s) to either CO_2 (filled symbols) or air (open symbols), and were retested at the indicated times (min, abscissa). Baseline means were 13.19 ± 1.09 and 11.88 ± 0.99 (s, air vs. CO_2, respectively) in naive rats; 11.22 ± 0.90 and 11.54 ± 1.00 (s, air vs. CO_2, respectively) in handled rats and did not differ between gas or handling groups, Fs < 1.31, $p > 0.05$. Antinociceptive scores (%MPE, ordinate, mean ± SEM) are shown. A two-factor (gas, handling) repeated-measures (time) ANOVA showed a significant main effect of gas, $F(1, 410) = 18.91$, $p < 0.001$, but failed to show a main effect of handling, $F(1, 41) = 0.29$, $p > 0.05$. No interactions were observed, Fs < 0.96. $^{**}p < 0.01$ for the difference between air vs. CO_2 in naive animals at the same time. $^{+,++}p < 0.05, 0.01$ for the difference between air vs. CO_2 in handled animals at the same time.

DISCUSSION

In an effort to organize proposed mechanisms of stress-induced analgesia, Watkins and Mayer (60) classified endogenous analgesia systems as either opiate or nonopiate, and either hormonal or neural [but see also Watkins et al. (61)]. Based upon this classification scheme, the novel form of CO_2-induced analgesia following recovery from anesthesia has characteristics of a nonopioid, hormonal response [(49); and see Watkins and Mayer (60)]. It has been previously reported that CO_2 induces a mild antinociceptive response during continuous inhalation of low concentrations of the gas (22,27,57), and following recovery from anesthesia associated with inhalation of higher concentrations of the gas (49). In both cases,

FIG. 3. CO_2-induced antinociception in restrained and naive rats as assessed by the hot plate (A, top) and tail flick (B, bottom) nociceptive methods. On the test day, animals were tested for baseline antinociception and were then either restrained as described (squares) or returned to their home cage (unrestrained controls, circles), were exposed (30 s) to either CO_2 (filled symbols) or air (open symbols), and were retested at the indicated times (min, abscissa). Hot plate baseline means were 13.78 ± 0.99 and 13.80 ± 0.94 (s, air vs. CO_2, respectively, n = 10) in naive rats; 14.64 ± 1.04 and 13.05 ± 1.13 (s, air vs. CO_2, respectively, n = 11) in restrained rats and did not differ between gas or restraint groups, Fs < 0.58, $p > 0.05$. Antinociceptive scores in hot plate-tested animals (%MPE, ordinate, mean ± SEM, n = 10–11) are shown (Fig. 3A). A two-factor (gas, restraint) repeated-measures (time) ANOVA showed significant main effects of restraint, $F(1, 38) = 4.65$, $p < 0.05$, and gas, $F(1, 38) = 16.19$, $p < 0.0005$, two-way interactions between restraint and time, $F(3, 114) = 7.02$, $p < 0.0005$, and gas and time, $F(3, 114) = 4.55$, $p < 0.005$, but failed to show an interaction between gas and restraint, Fs < 1.17, $p > 0.05$. Tail flick baseline means were 3.52 ± 0.12 and 3.20 ± 0.07 (s, air vs. CO_2, respectively, n = 11–15) in naive rats; 3.33 ± 0.16 and 3.39 ± 0.11 (s, air vs. CO_2, respectively, n = 10–12) in restrained rats and did not differ between gas or restraint groups, Fs < 2.46, $p > 0.05$. Antinociceptive scores in tail flick-tested animals (%MPE, ordinate, mean ± SEM) are shown (Fig. 3B). A two-factor (gas, restraint) repeated-measures (time) ANOVA showed a significant main effect of gas, $F(1, 44) = 7.74$, $p < 0.01$, and time, $F(3, 132) = 3.67$, $p < 0.05$, two-way interactions between restraint and time, $F(3, 132) = 2.76$, $p < 0.05$, and gas and time, $F(3, 132) = 8.91$, $p < 0.00005$, and a three-way interaction involving restraint, gas, and time, $F(3, 132) = 4.75$, $p < 0.005$. $^{*,**}p < 0.05, 0.01$ for the difference between air vs. CO_2 in animals within the same restraint group at the same time. $^{\#,\#\#}p < 0.05, 0.01$, respectively, for the difference between restraint vs. naive treatments in animals exposed to the same gas at the same time.


Naive (Air)
Naive (CO2)
Restr. (Air)
Restr. (CO2)
*,** p<0.05, 0.01 Air vs. CO2
#,## p<0.05, 0.01 Restr. vs. Naive
% MPE
TIME (Min)


Naive (Air)
Naive (CO2)
Restr. (Air)
Restr. (CO2)
*,** p<0.05, 0.01 Air vs. CO2
p<0.01 Restr. vs. Naive
% MPE
TIME (Min)

it is presumed that the CO_2-induced response is a stress-mediated event; however, the mechanism by which CO_2 causes a moderate reduction in pain perception is unknown. To further characterize this antinociceptive response, several methodological and biological parameters were evaluated, and the interaction between CO_2 and restraint (another well-known activator of stress-induced analgesia) was explored.

It is well known that diurnal variability in pain sensitivity exists in basal response (16,42,43,48) and during conditions of enhanced antinociception (18,21,47,52). Thermal, basal response latency is greater during nocturnal portions of the daily cycle, possibly associated with elevated nocturnal levels of opioid peptides (18,32,33,51). Thus, it is not uncommon to use animals housed under reversed, light-cycle conditions in nociceptive testing protocols (14,24,31,58). In the present study, it was hypothesized that testing animals during the dark portion of their daily cycle would increase basal antinociceptive response and would optimize the antinociceptive sensitivity following inhalation of CO_2. In contrast, the study showed that hot plate baseline responses and antinociceptive responses following inhalation of CO_2 are not altered by housing animals under different light-cycle conditions. Although, similar to previous studies (32,51), our results show that nociceptive baselines in light-cycled animals tended to be lower than those in dark-cycled animals ($p = 0.09$). These findings are consistent with the previous observation that opiate receptors are not obligatory for the CO_2-induced effect (49) because, unlike opiate analgesia (34), the CO_2-induced antinociception was not enhanced in reversed-light cycle animals. Subsequent studies were performed in animals housed under normal light cycle conditions.

Habituation of animals to testing procedures for 3 to 5 days is common in pain sensitivity studies (4,5,11,12). Calcagnetti and Holtzman (13) showed that habituation to testing regimens decreases vocalization, defecation, and struggling during nociceptive testing and optimizes stress-induced analgesic responses. In the present study, habituation seemed to diminish defecation and struggling during testing; in contrast, habituation did not alter the CO_2-induced response. Besides being habituated to the testing apparatus, animals were habituated to the brief immobilization that routinely occurs during nociceptive testing. It is interesting that during some studies involving immobilization [a stressor known to evoke an opiate-mediated form of analgesia, see Amir and Amit (1)] it was found that habituation to immobilization diminished immobilization-induced stress analgesia (11) and attenuated immobilization-induced potentiation of opiate analgesia (10). In contrast, the present finding that habituation to testing and immobilization does not alter the CO_2-induced response suggests that the mechanism of CO_2 -induced antinociception is fundamentally different from the mechanism(s) involved in immobilization analgesia. These results are consistent with the previously reported finding that CO_2-induced antinociception is not dependent on opiate mechanisms (49).

Age-related changes in the antinociceptive responses to a variety of stressors (23,40,41) and to morphine (35) have been reported. Antinociceptive sensitivity is highest in young adult animals (40); and, most studies indicate that the magnitude of analgesia following exposure to the stressor or morphine diminishes in aged animals [but see Hamm and Lyeth (28)]. It was hypothesized that larger, more mature animals that had been naturally exposed to more environmental stressors would exhibit a higher or less variable CO_2-induced effect. In contrast, the antinociceptive response following CO_2 exposure was similar between animals that were less than 300 g (less than 8 weeks of age) and animals that were greater than 300 g (up to 8 months of age). These data support the findings of Kramer and Bodnar (40) who found that rats between 4 and 19 months of age responded similarly to continuous cold-water swim analgesia (CCWS).

Gender differences in pain perception have been identified in a variety of analgesia models (37,39,53,54). In most cases, females were found to elicit less analgesia compared to males following stressor or opiate exposure; and, estrous cyclicity was found to influence analgesic responses in some (6,19) but not other studies (53). Because the mechanisms of CO_2-induced and CCWS analgesia are similar [nonopiate, hormonal; see Mischler et al. (49), and others (8,26,56)], and because gender differences were observed in the CCWS model (53,54), the effect of gender on the CO_2 antinociceptive response was studied. In contrast to the influence of gender on CCWS analgesia, a difference between male and female antinociception following exposure to CO_2 was not found. These results indicate that both male and female animals are capable of producing similar analgesic responses following this stressor, a characteristic not seen in CCWS (53,54) or other stress-induced analgesias (15,19,36). These findings suggest that the antinociception following exposure to CO_2 or CCWS are different. Moreover, because the magnitude of the antinociceptive response following CO_2 is moderate in comparison to other stress-analgesia models, these findings may suggest that those variables that are important for gender differences following severe stressor exposure are not important in the production of moderate, stress-induced antinociception. Interestingly, during the present experiments, it was also observed that 30% of females and only one male displayed persistent jumping responses on the hot plate. In all nociceptive trials, animals that jumped were, by definition, discarded because it was uncertain whether jumping was indicative of pain perception and because this jumping response obscured determination of latency to lift and lick or multistamping. Others have described female hyperresponsiveness in nonthermal nociceptive tests (46,53); this is the first suggestion that female hyperresponsiveness may also occur on thermal tests.

Restraint stress has been shown to induce an antinociceptive response that is dependent upon endogenous opiate activity (1,38), is abolished by hypophysectomy (2) and is attenuated by habituation to the restraint paradigm (11). Taken together, these studies suggest that the mechanism of restraint analgesia may involve pituitary-derived endogenous opiates. Previous results from this laboratory showed that CO_2 also induces a pituitary-dependent, antinociceptive response; although the CO_2-induced effect is not mediated by an opiate mechanism (49). Furthermore, it is well documented that restraint stress potentiates opioid-induced analgesia (3,4,10,13), and that the potentiation is centrally mediated (5). Thus, it was hypothesized that restraining animals prior to CO_2 exposure would result in a restraint-induced analgesic response that would overlap and/or interact with the CO_2-induced antinociception. The present results suggest that the effect of restraint on CO_2-induced antinociception is dependent on the nociceptive test employed. Although both restraint and inhalation of CO_2 induced an antinociceptive response on the hot plate test, a restraint by CO_2 interaction was not observed. In contrast, on the tail flick test, CO_2-restraint interactions caused time-dependent changes in the nociceptive response. Though CO_2 induces antinociception on both the hot plate and tail flick tests, disparity in CO_2 response between nociceptive tests following restraint implies differences in the mechanism of antinociception following CO_2 alone vs. in combination with re-

straint. Others have suggested that test-specific differences in antinociceptive responses provide evidence for divergence in pain-suppression mechanisms originating from the periaqueductal gray (30,50).

The statistical interaction observed between CO_2, restraint, and time, in the tail flick data, supports two opposing hypotheses, depending on the test interval following gas exposure. It is possible that both inhibition and synergism are occurring between the presently studied opiate and nonopiate forms of analgesia on the tail flick test. At 10 min following gas exposure, restraint negatively inhibited the CO_2-induced analgesia. In contrast, at the 40 min time point, the tail flick data are reminiscent of the restraint-induced potentiation of opiate analgesia; and, at this time point it appears that restraint (an opiate mechanism of analgesia) can be synergistic with CO_2 (a nonopiate mechanism of analgesia). An alternative explanation of the tail flick data could be that the two analgesic mechanisms invoked in these experiments are separate and distinct, but follow two separate time courses. The measured antinociceptive response at each time point could depict that system that is most active at that particular time. Antagonist studies might be able to separate the two events. Additional studies investigating the importance of thermal intensity may also be valuable in characterization of CO_2-restraint interactions on the hot plate and tail flick tests.

In conclusion, inhalation of 100% CO_2 induces an antinociceptive response under a wide variety of methodological and biological conditions. Neither methodological manipulation of the testing paradigm nor biological differences between animals affect the CO_2-induced response suggesting that CO_2-induced antinociception is not like other forms of nonopiate, hormonal analgesia. Also, special methodological or biological manipulations are unnecessary for the study of CO_2-induced antinociceptive events. Finally, restraining animals prior to exposing them to CO_2 or air, had no effect on the CO_2-induced response in animals tested by hot plate methods; but, in animals tested by the tail flick method, restraint can, at different time points, both abolish and synergize with the CO_2-induced antinociceptive response. Taken together, the present experiments demonstrate that CO_2 antinociception is a repeatable and novel form of stress-induced analgesia.

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