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Effects of Chlordiazepoxide on Opioid-Induced Antinociception and Respiratory Depression in Restrained Rats

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VERBORGH, C., R. DE COSTER, J. D'HAESE, F. CAMU AND T. MEERT. *Effects of chlordiazepoxide on opioid-induced antinociception and respiratory depression in restrained rats.* PHARMACOL BIOCHEM BEHAV **59**(3) 663–670, 1998.—This study investigates the influence of possible stress due to housing in Bolman cages on antinociception and on respiratory depression following opioid administration. To evaluate the functional role of this stressor and to modulate it, rats were subcutaneously pretreated with the anxiolytic chlordiazepoxide (CDP; 10 mg/kg) or saline (SAL) before the immobilization in the Bolman cages and before the intravenous administration of small doses of morphine (MOR), sufentanil (SUF), or vehicle (VEH). Antinociception, respiratory impairment and stress were evaluated by means of the tail-flick latency, blood gas analysis, and serum corticosterone (CS), adrenocorticotropic hormone (ACTH), and prolactin (PRL) determinations. The results demonstrated that 10 mg/kg CDP did not alter the antinociceptive effects of low doses of morphine and sufentanil. CDP pretreatment differentially affected the various blood gas parameters. Compared to vehicle pretreatment, there was a larger decrease in PaO_2 following MOR and SUF in the CDP-pretreated rats. The effects were most pronounced at the lowest doses of both opioids. A CDP potentiation was also observed for the short-lasting raises in $PaCO_2$ with the lowest concentrations of the opioids. At higher concentrations of the opioids, CDP was without any effect. With regard to the stress hormones, immobilization and an intravenous injection resulted in increases in CS and PRL in both CDP- and VEH-pretreated rats. ACTH did not change in these controls. SUF prevented the CS raises independent of a CDP pretreatment, while ACTH only increased in the SUF plus CDP groups, pointing to a stress-reducing effect of SUF. Also, MOR without CDP prevented the increases in CS, but the opioid intrinsically increased ACTH. These results indicate that restraint in Bolman cages in the present setup, with animals recovering for several hours in these cages after being equipped with an arterial catheter, is stressful but without any significant effect on the opioid-induced antinociception. Pretreatment with an anxiolytic benzodiazepine only minimally affected the outcome of the opioids on respiratory depression and pointed to a stress-reducing effect of low doses of the opioids, especially sufentanil. © 1998 Elsevier Science Inc.

Morphine Sufentanil Restraint stress Stress-induced analgesia Adrenocorticotropic hormone Corticosterone Prolactin Chlordiazepoxide

RESPIRATORY depression upon intravenous administration of an opioid is a well-known phenomenon. For studying the relation between blood gas deviations and antinociception using the tail-flick latencies, the Bolman cage is a useful device, because it allows simultaneous sampling of blood and antinociceptive measurements by immersion of the free-hanging tail into warm water without further handling the animals. The influences of restraint stress on antinociception have already been reported, although most articles deal with different forms of restraint, such as taping the upper body and forelimbs (36), the immobilization of the animal in Plexiglas cylinders (13) or a gentle holding of the rodents with thumb and index finger (33). Reports on the effects of restraint in Bolman cages on antinociception are scarce, and the influence

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of housing in Bolman cages on opioid-induced respiratory depression has not been documented so far.

Stress, painful stimuli and hypnotics, which are often part of a laboratory setup, may considerably alter respiratory variables. For instance, we observed a biphasic dose–response curve in $PaCO_2$ and higher PaO_2 values with systemic administered morphine and sufentanil in immobilized awake rats (35). Especially low doses of the opioids appeared to improve the respiratory functioning in these stressful conditions. It was unclear whether these effects on ventilation were due to an increased respiratory rate or stress. Therefore, the present study was designed to evaluate the effects of systemic administration of low doses of morphine and sufentanil on blood gas variables, antinociception, and stress hormones in rats subjected to immobilization stress in Bolman cages. To study the impact of a reduction of the stress component, the benzodiazepine chlordiazepoxide was included in the experimental setup.

METHOD

Animal Preparation

Approval from the Institutional Animal Care and Use Committee was obtained to perform the experiments described. Seventy-one naive male Wistar rats weighing 220–260 g were chosen, because this strain produces the most robust stress effect (37). They were housed in a room with controlled temperature (21 ± 1°C) and humidity (65 ± 5%) and light–dark cycles. The animals were anesthetized with ether. After preparation of the left femoral artery, a polyethylene catheter (PE 50) was inserted into the proximal end for 3 mm. The rats were then installed in Bolman cages to emerge from anesthesia. The longitudinal metal rods of the cages were adjusted as to minimize impairment of breathing movements, as any obstruction to normal thoracic or abdominal expansion would seriously alter blood gas variables (16). The animals were kept in these cages during the entire observation period. Before and during the experiments the femoral lines were kept patent using 200-μl injections of a solution of Heparin sodium (100 U/ml). All observations and blood sampling was done between 10.00 and 12.00 h.

Assessment of Respiratory Function

Arterial blood samples (200–250 μl) were collected from the femoral cannulla into 1-ml heparinized syringes. Blood samples were kept on ice until analysis. The pH, PaO_2, $PaCO_2$, and oxygen saturation ($SatO_2$) were determined using a blood gas analyzer (ABL3 Radiometer Copenhagen®).

Assessment of Antinociception

The time of the withdrawal of the tail (tail-flick latency: TFL) being immersed for 5 cm into a bath filled with demineralized water kept constant at 55 ± 1°C (Julabo Labotechnik®) was recorded to the nearest 0.01 s. To minimize tissue damage on repetitive measurements, a cutoff time of 10 s was used. A TFL of >6 s never occurred in control animals and was adopted as a criterion of mild antinociception. A TFL of ⩾10 was used as a criterion for deep surgical antinociception (19).

Determination of Stress Hormones

Blood samples were collected in 1.5-ml polypropylene siliconized microcentrifuge tubes and kept in ice until cooled centrifugation (20 min at 10,000 ± 2% rpm). Serum (500 μl) was transferred (Pipetman GILSON) into new tubes and immediately frozen (−20°C) until assay.

Plasma corticosterone levels were determined directly after dilution (1/400 or 1/800) in phosphate buffer, pH 7.6, containing 9 g/l NaCl and 0.1% gelatin. The diluted serum was heated at 98°C for 10 min to denature the radioimmunoassay. It is expressed as nmol/l. ACTH and prolactin were measured by commercially available kits, ACTH radioimmunoassay (Nichols Institute Diagnostic, San Juan Capistrano, CA), and an homologous enzyme-immunometric assay (Milena® rat prolactin, Diagnostic Product Corporation, Humbeek, Belgium). ACTH and prolactin levels are expressed as pg/ml and ng/ml, respectively.

Experimental Design

Opioid injection via a lateral tail vein was at least 3 h following the ether anesthesia and upon normalization of the blood gas analysis variables. Two groups were studied; one group of 35 animals was premedicated with 10 mg/kg chlordiazepoxide administered subcutaneously 1 h before the opioid administration (called the CDP or pretreated group), while the other group received saline (called the SAL or nonpretreated group). In both groups, subgroups of five animals received increasing doses of sufentanil or morphine, while five more animals were injected with saline and served as controls. The doses injected were 0.00016, 0.00031, and 0.00063 mg/kg sufentanil or morphine hydrochloride at 0.31, 0.63, and 1.25 mg/kg. Tail-flick latency and blood gasses were assessed before and at 2, 5, 10, 15, 30, and 60 min following administration of the opioid. Blood samples for hormone level determination were taken before and at 10, 30, and 60 min after the opioid injection. Tail-flick latencies were measured after blood sampling to avoid the influence of the thermal stimulation on the blood gas values. Approximately the same volume blood lost by sample collection was replaced by saline to keep circulating volume steady.

Data Analysis

The postdrug response (TFL) was expressed as %MPE where %MPE=(TFL max − TFL preinjection)/(cutoff time − TFL preinjection). Data on blood gas variables are expressed as % change compared to preinjection values. Areas under curve (AUC) were calculated. Statistical analysis included analysis of variance for repeated measurements and significant differences between the groups (pretreated or not) or between different opioid drug concentrations and saline were revealed by means of the Mann–Whitney U-test (two-tailed). Differences with respect to preinjection values were evaluated using the Wilcoxon test (two-tailed). A p-value <0.05 was considered significant.

Chemicals

Sufentanil citrate and morphine hydrochloride were dissolved in saline in different recipients in such a way that injection of a certain dose always consisted of 250 μl. Chlordiazepoxide hydrochloride was dissolved in saline and used in a concentration of 10 mg/kg.

RESULTS

TFL vs. Time Analysis

Mean (SEM) baseline latencies for the TFL averaged 2.62 (0.98) s (range: 1.61–4.2 s) in the saline-pretreated group and

1.75 (0.33) s (range: 1.26–2.14) in the chlordiazepoxide-pretreated group (Fig. 1). Intravenous injection of vehicle did not produce a significant difference compared to baseline in the saline-pretreated control group, whereas in the chlordiazepoxide-pretreated group the TFL increased significantly immediately following the IV vehicle injection. TFL significantly increased already 2 min after injection of either dose of sufentanil and morphine. The maximum antinociceptive effect was reached within 15 min for each dose of sufentanil or morphine used, while onset of maximal effect was clearly dose dependent in the rats treated with sufentanil. Increases in TFL stayed significant compared to baseline after all doses of sufentanil and morphine during 60 min. In all test conditions using sufentanil and in all conditions with the two highest doses of morphine, TFL values > 6.0 s were reached. At 1.25 mg/kg morphine, the TFL > 6.0 remained present over the entire observation period. Several doses of morphine and sufentanil increased the TFL values above the vehicle control levels. Comparing similar doses of morphine and sufentanil in saline and chlordiazepoxide-pretreated rats, no differences in antinociception were observed, except for 1.25 mg/kg morphine. At this dose, the duration of activity of the chlordiazepoxide-pretreated rats was shorter than the activity of the corresponding saline-pretreated animals.

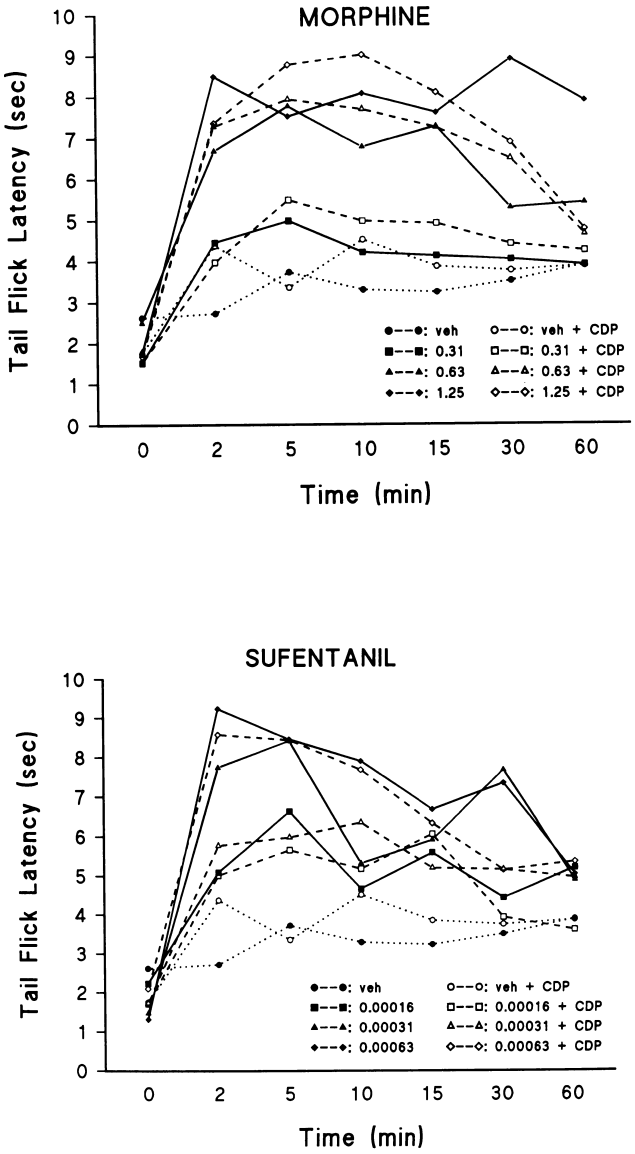

FIG. 1. Tail-flick latencies over time. Given for the various doses tested of morphine (upper panel) and sufentanil (lower panel) are the mean tail-flick latency values of five rats per drug condition. The animals were pretreated subcutaneously with either saline or 10 mg/kg chlordiazepoxide (+ CDP). The opioids or vehicle were injected intravenously.

Dose vs. Antinociceptive Effect Analysis

Intravenous injection of 0.00016 to 0.00063 mg/kg sufentanil or 0.31 to 1.25 mg/kg morphine resulted in a dose-dependent increase in the tail-flick latency as reflected in the % MPE (Fig. 2) in both groups. The dose–response curves were linear in a semilogarithmic plot for both sufentanil and morphine. Pretreatment with or without chlordiazepoxide did not affect the outcome. Differences from intravenous vehicle were present with doses ⩾0.63 mg/kg morphine and indepen-

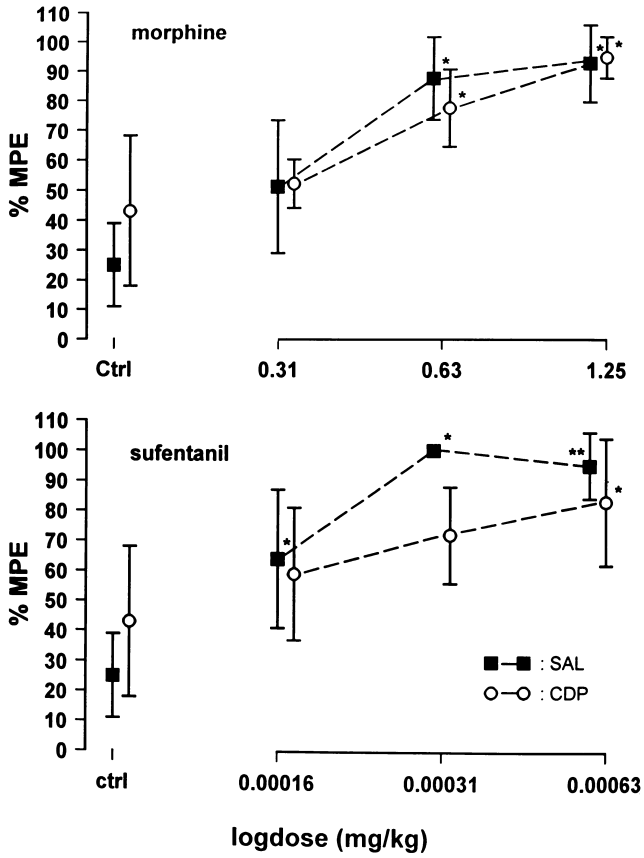

FIG. 2. Antinociceptive effects of intravenously administered morphine and sufentanil in the tail-flick latency test in rats in terms of the percentage maximal possible effect. Given for the different doses are the mean (±SEM) values of five rats per treatment condition. The animals were either pretreated with saline (closed squares) or 10 mg/kg chlordiazepoxide (open circles). Differences with the corresponding intravenously administered vehicle controls (ctrl) were evaluated using the Mann–Whitney U-test (two-tailed; $*p < 0.05$; $**p < 0.01$).

dent of the pretreatment. In the vehicle-pretreated rats, all doses of sufentanil resulted in a significant increase in the %MPE. In the chlordiazepoxide-pretreated rats, differences with the vehicle controls were only statistical significant at 0.00063 mg/kg sufentanil. Globally, the dose–response curves of morphine did not deviate from those of sufentanil, reflecting the comparable potency of the doses selected.

Additional observations indicated that the chlordiazepoxide-pretreated rats were more flaccid than their saline-pretreated counterparts; no other behavioral signs were detected.

Blood Gas Variable vs. Time Analysis

Effects on $PaCO_2$. The intravenous injection of 0.00063 mg/kg sufentanil and 0.63 and 1.25 mg/kg morphine resulted in a brief but significant period of hypercapnia shortly after administration in both the chlordiazepoxide- and saline-pretreated groups (Fig. 3; upper panel). In terms of the area under the curve during the entire measurement period (Fig. 4; upper panel), a significant increase in $PaCO_2$ was only observed with 0.63 mg/kg in the chlordiazepoxide-pretreated group, especially due to the large interindividual variability in the second part of the measurement period. There were no statistical differences between the various comparable groups pretreated with or without chlordiazepoxide.

Effects on PaO_2. In the saline-pretreated rats, none of the doses of morphine or sufentanil resulted in a decrease in PaO_2 below the vehicle control level at any of the measurement periods (Fig. 3; middle panel). Differences from the preinjection control baseline were present at 1.25 mg/kg morphine and 0.00031 and 0.00063 mg/kg sufentanil in the chlordiazepoxide group. Following chlordiazepoxide pretreatment, there was an increase in the PaO_2 over the entire measurement period in the IV vehicle-treated rats. For all tested doses of the opioids, the PaO_2 decreased below the corresponding vehicle level. Differences between vehicle and chlordiazepoxide-treated groups were only seen with 0.31 mg/kg morphine. In terms of the area under the curve, all opioid concentrations reduced the PaO_2 below the vehicle level in the chlordiazepoxide-pretreated rats (Fig. 4; middle panel). In the saline-pre-

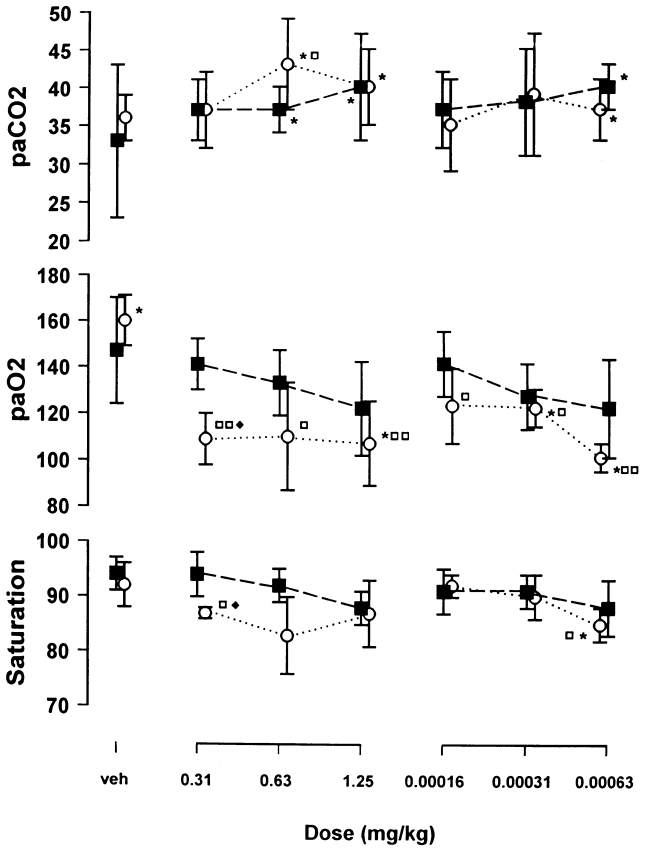

FIG. 3. Effects of intravenously administered morphine and sufentanil on blood gas variables in rats. Given for the different doses of the opioids and vehicle are the mean (±SEM) maximal effects of five rats per treatment condition on $PaCO_2$ (in mmHg), on PaO_2 (in mmHg), and on oxygen saturation (%). The animals were either pretreated with saline (closed squares) or 10 mg/kg chlordiazepoxide (open circles). Shifts between experimental conditions were evaluated using the Mann–Whitney U-test and the Wilcoxon Signed-Rank test (two tailed). The asterisks (*) indicate differences with the preinjection baselines; the open squares (□) refer to differences with the intravenous vehicle conditions and the closed diamonds (◆) refer to differences between the saline and the chlordiazepoxide pretreatment. One symbol: $p < 0.05$; two symbols: $p < 0.01$).

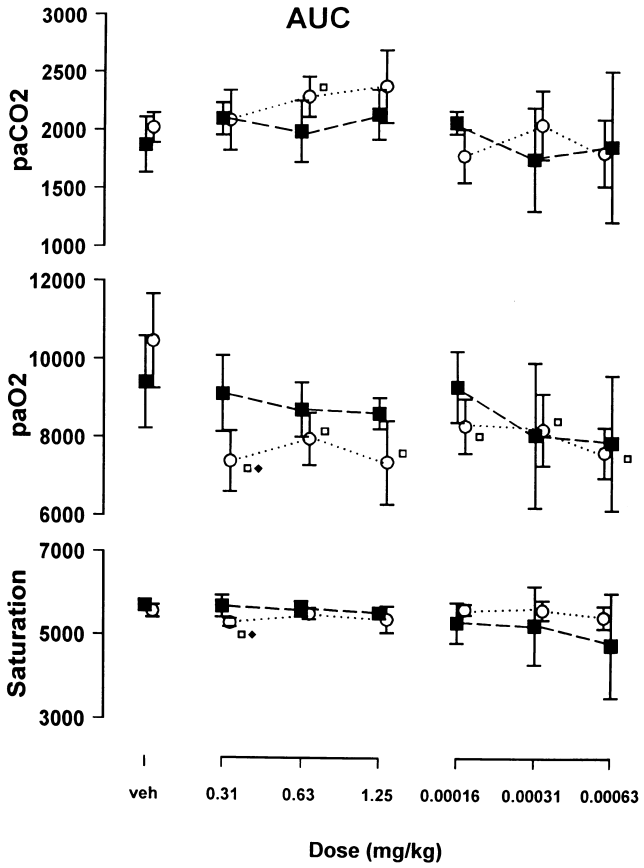

FIG. 4. Effects of intravenously administered morphine and sufentanil on blood gas variables in rats in terms of the area under the curve obtained during the 60 min observation period. Given for the different doses of the opioids and vehicle are the mean (±SEM) values of five rats per treatment condition on $PaCO_2$, PaO_2, and on oxygen saturation. The animals were either pretreated with saline (closed squares) or 10 mg/kg chlordiazepoxide (open circles). See also legend to Fig. 3.

treated rats, no differences were present. Also here, there was a difference between 0.31 mg/kg morphine with or without chlordiazepoxide.

Effects on oxygen saturation. The effects on oxygen saturation were very limited. In the vehicle-pretreated rats there were no significant reductions either in terms of the maximal shift at a moment (Fig. 3; lower panel), or in terms of the area under the curve during the entire measurement period (Fig. 4; lower panel). In the chlordiazepoxide-pretreated rats, a dose of 0.00063 mg/kg sufentanil reduced the mean $SatO_2$ values below the preinjection baseline at 5 min after treatment, and at that point there was also a difference with the vehicle-treated group. For morphine, the lowest dose of 0.31 mg/kg resulted in differences with the corresponding benzodiazepine-pretreated vehicles and with the corresponding saline-pretreated rats.

Stress Hormone Analysis

Corticosterone. Before the intravenous injection of sufentanil, morphine or vehicle, there were no significant differences between the different groups, although the corticosterone levels in the chlordiazepoxide-pretreated animals were somewhat lower than those of the saline-pretreated groups. The intravenous injection of vehicle resulted, compared to the preinjection baseline values, in a significant raise in corticosterone in both the chlordiazepoxide- and the saline-pretreated rats up to 60 min (Fig. 5). Also, the injection of morphine resulted in a dose-dependent increase in corticosterone in both pretreatment groups (Fig. 5; upper panel). Increases above baseline were significant at 0.63 and 1.25 mg/kg morphine in the chlordiazepoxide group and at 1.25 mg/kg morphine in the saline-pretreated rats. At 0.31 mg/kg morphine, the mean corticosterone levels were lower than those of the corresponding vehicles and a difference between both pretreatment groups was present. Similarly, at 0.63 mg/kg morphine, there was a difference between the saline- and the chlordiazepoxide-pretreated groups and in the saline-pretreated animals, a difference with the vehicle controls was also present. Intravenous sufentanil injections did not raise the corticosterone levels above the preinjection baseline values. In all conditions, the mean corticosterone levels were lower than those observed after intravenous vehicle (Fig. 5; lower panel).

Adrenocorticotropic hormone. Before the intravenous injections, the mean ACTH levels were somewhat lower in the chlordiazepoxide-pretreated groups compared to the saline-pretreated groups, but no significant differences were noted. The intravenous injection of vehicle was without any major effect on the average basal ACTH levels. The injection of intravenous morphine resulted in a significant increase in ACTH that was not prevented but even significantly enhanced by the chlordiazepoxide pretreatment. The increases reached a maximum differentiation at 30 min after injection (Fig. 6; upper panel). Injection of sufentanil increased ACTH levels only in chlordiazepoxide-pretreated animals (Fig. 6; lower panel).

Prolactin. Prolactin levels were variable between different animals in both the chlordiazepoxide- and the saline-pretreated groups, and increases were only observed in part of the animals. Table 1 presents the number of animals per treatment condition in which the individual prolactin level was increased with >20% above the baseline value at both 30 and 60 min after the intravenous treatment with either vehicle or one of the doses of the opioids. Globally, there is an increased number of rats with elevated prolactin levels in the saline pre-

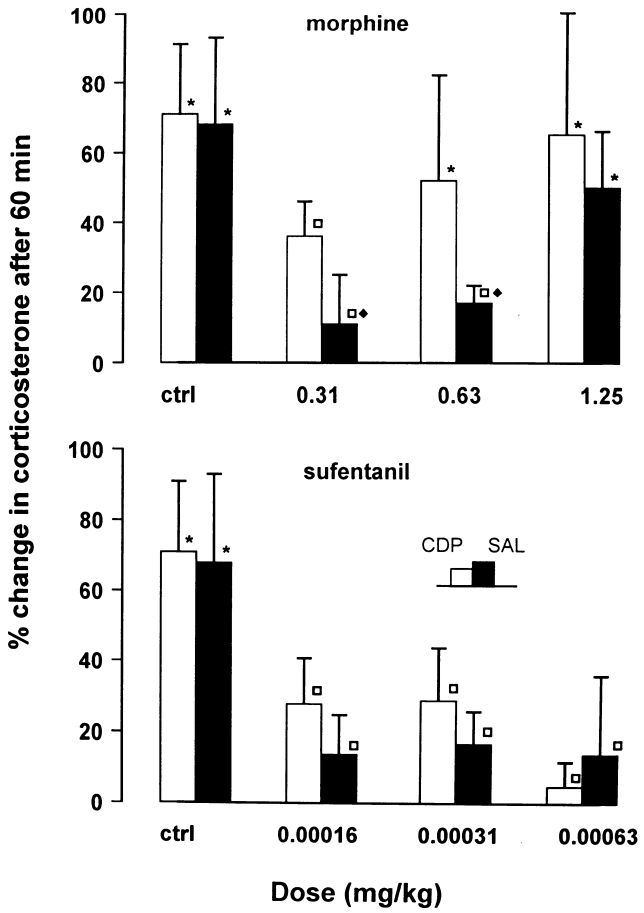

FIG. 5. Effects of intravenously administered morphine and sufentanil on corticosterone levels in rats. Given for the different doses of the opioids and vehicle are the mean (±SEM) percentages changes from baseline of five rats per treatment condition at 60 min after the intravenous injection. The animals were either pretreated with saline (closed bars) or 10 mg/kg chlordiazepoxide (open bars). See also legend to Fig. 3.

treated rats compared to the chlordiazepoxide pretreatments. The differences between the pretreatment groups became significant after 30 min in the intravenous vehicle group and the 0.00016 mg/kg sufentanil group.

DISCUSSION

The present experiment was performed to evaluate the effects of restraint stress in Bolman cages on antinociception and respiratory depression following intravenous administration of equipotent low doses of sufentanil and morphine. To reduce possible stress effects, part of the animals were pretreated with an active but not motor disruptive concentration of the benzodiazepine chlordiazepoxide. The results of the study can be summarized as follows: (a) subcutaneous pretreatment with 10 mg/kg chlordiazepoxide did not alter the antinociceptive properties of the low doses of the opioids. (b) The low doses of the opioids resulted in a short-lasting respiratory depression, as evidenced by a rapid increase in $PaCO_2$, without major effects of PaO_2 and oxygen saturation. Chlordiazepoxide pretreatment did not affect the hypercapnic ef-

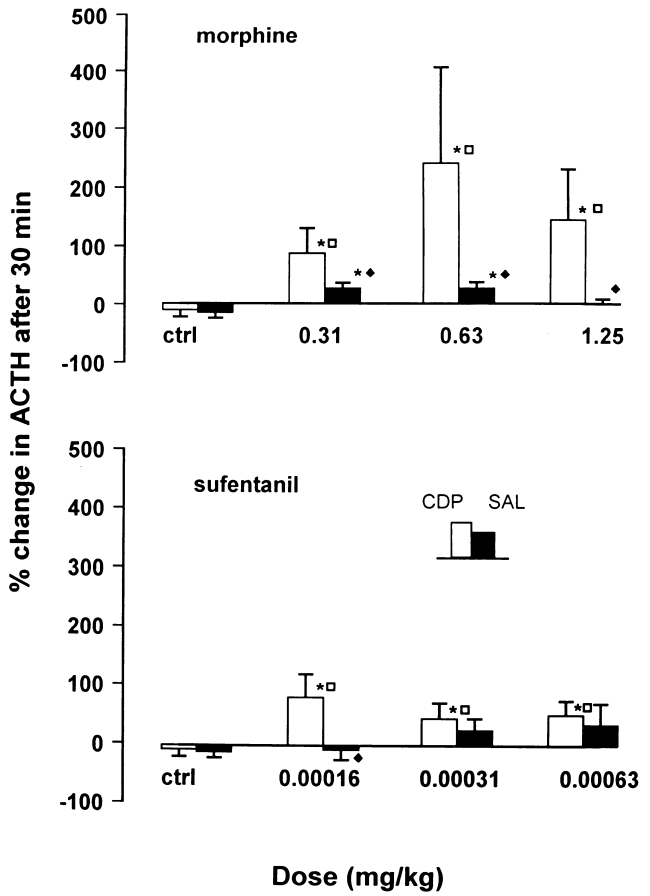

FIG. 6. Effects of intravenously administered morphine and sufentanil on ACTH levels in rats. Given for the different doses of the opioids and vehicle are the mean (±SEM) percentages changes from baseline of five rats per treatment condition at 30 min after the intravenous injection. The animals were either pretreated with saline (closed bars) or 10 mg/kg chlordiazepoxide (open bars). See also legend to Fig. 3.

TABLE 1

RAISE IN PROLACTIN AS A FUNCTION OF TREATMENT

		30 Min		60 Min	
Compound	Dose	Saline	CDP	Saline	CDP
Vehicle	(saline)	4/5	0/5*	2/5	1/5
Morphine	0.31 mg/kg	3/5	1/5	2/5	0/5
	0.63 mg/kg	2/5	0/5	2/5	2/5
	1.25 mg/kg	3/5	2/5	3/5	2/5
Sufentanil	0.00016 mg/kg	4/5	0/5*	5/5	2/5
	0.00031 m/kg	2/5	0/5	4/5	1/5
	0.00063 mg/kg	2/5	2/5	2/5	3/5

Given for the animals tested at each treatment condition are the number of rats having an increase in basal prolactin levels with more than 20% at either 30 or 60 min after the intravenous injection with either vehicle or one of the doses of morphine or sufentanil. Before the intravenous treatment, the animals were subcutaneously pretreated with either saline or 10.00 mg/kg chlordiazepoxide (CDP). Differences between the saline and the CDP-pretreated groups were evaluated using the Fisher Exact probability test (two-tailed); $*p < 0.005$).

fects of the opioids but the benzodiazepine clearly increased the opioid-induced hypoxia. The effects on oxygen saturation were also minimal. (c) With regard to the stress hormones, immobilization in the Bolman cages and an intravenous treatment with saline resulted in a gradual increase in corticosterone over time, an increase in prolactin within 30 min after treatment and no major effects on ACTH. With the opioids, differential effects were observed. With morphine, there was reversed relationship between the doses and the corticosterone concentration; the lower the dose, the lower the concentration of corticosterone. Also for sufentanil, the corticosterone levels were lower than those in the vehicle control group. Pretreatment with chlordiazepoxide diminished the corticosterone-reducing properties of morphine while having no effects at all on sufentanil. These results indicate that low doses of opioids, and especially sufentanil, have intrinsic stress-reducing properties that can be overcome by a treatment with a benzodiazepine. With regard to ACTH, the two lowest doses morphine resulted in a significant increase of the ACTH levels above the vehicle control level. Chlordiazepoxide pretreatment significantly increased the ACTH levels in all opioids groups; the shifts in the morphine-treated rats were more pronounced than those in the sufentanil-treated animals. With regard to prolactin, the interindividual variability was enormous, but there was a tendency to a reduced number of animals with pronounced increases in prolactin after chlordiazepoxide. The opioids had no clear effects on individual prolactin levels.

The relationships between stress, benzodiazepine, opioids, and hormonal shifts with regard to antinociception are already complex, but their functional interactions with respect to respiratory depression are even more complicated. Several reports deal with the effects of immobilization or restraint stress, and the hormonal changes it causes, on opioid-induced antinociception. Immobilization or restraint stress produces antinociception with elevated plasma levels of ACTH and beta-endorphin (36), stimulation of proopiomelanocortropin mRNA levels in both lobes of the pituitary (25) and Met-enkephalin–like immunoreactivity in hypothalamus and thalamus (23). Restraint stress also increases the antinociceptive properties of systemic administered opioids (3,24) and intracerebroventricularly injected enkephalins (4). In our experiments, the restraint in the Bolman cages did not result in a stress-induced antinociception in the vehicle-treated rats. Also, the pretreatment with an active anxiolytic dose of the benzodiazepine chlordiazepoxide (it reduced the basal concentration of corticosterone, for instance, before the intravenous treatments and reduces prolactin over time), which did not have any intrinsic antinociceptive properties here (see chlordiazepoxide-pretreated vehicle controls) and in other studies (32), did not modulate the antinociceptive properties of the low doses of morphine and sufentanil, pointing to a lack of any stress-induced sensitivity shift in the experimental setup. It might be that long-term immobilization in Bolman cages is just not stressful enough to produce stress-induced analgesia. As opposed to animals being taped or placed in a narrow cylinder, the animals in Bolman cages can still move their limbs and head, and to some degree the abdomen and the thorax. The latter is important with regard to respiratory functioning. Furthermore, in the present study, the animals emerged from anesthesia in the Bolman cages and inevitably

remained there for some time. Some degree of stress was, nevertheless, present, as evidenced by the relative higher values of corticosterone and ACTH at the beginning of the experiment compared to other baseline levels in restraint studies (1) and the gradual augmentations of both hormones over time; an effect also reported in other restraint models (6,34). The final hormonal shifts were, however, lower than those reported after 1 h of classical restraint (17,21). Also, the here-observed prolactin increases reflect a stress response in the rats (20).

Opioids are reported to interfere with stress hormones. Several reports demonstrated a stimulation of the hypothalamo–pituitary–adrenocortical (HPA) axis by morphine (27,31) and various mu-, kappa-, and delta- agonists (5,18,29), as well as an exaggerated HPA-axis activity to stress in acutely morphine treated rats (8). Subcutaneous morphine injections were also reported to provoke a raise in growth hormone and prolactin (5,31). One has to remark, however, that the doses of morphine used in these studies are much higher than those of the present experiments. In the present experiment, the low doses of 0.31 and 0.63 mg/kg morphine were able to prevent the stress-induced increases in corticosterone over time after an intravenous administration (Fig. 5) , pointing to a stress-reducing effect of morphine in this dose range (12). With sufentanil, there was, compared to the intravenously treated vehicle controls, a clear-cut reduction in the corticosterone increases over time. These data thus support the stress-reducing properties of sufentanil reported in other studies (11,22). As opposed to morphine, pretreatment with chlordiazepoxide did not suppress the antistress effects of the present doses tested of sufentanil. Furthermore, the combination of morphine plus chlordiazepoxide also resulted in increased levels of ACTH (Fig. 6). It is not clear at the present whether these higher levels of corticosterone and ACTH in the morphine plus chlordiazepoxide-treated rats are due to a reversal of the antistress effects of low doses of morphine with chlordiazepoxide or to an intrinsic increase of these hormones by the benzodiazepine itself, as reported elsewhere (9,10). Nevertheless, the combination of both compounds results in a clear potentiation with respect to the stress hormones.

The interactions between a benzodiazepine and an opioid are not limited to stress reactivity. Numerous reports deal with the interactions of benzodiazepines with opioids at spinal and supraspinal sites using various routes of administration and antagonistic (26,30) and potentiating interactions (7) are described. In our study, the selected dose of chlordiazepoxide did not have any effect on the opioid-induced antinociception. Chlordiazepoxide had clearly a depressing effect on respiration. When coadministered with low doses of the opioids, significant lower PaO_2 values were measured compared to the opioids alone (Figs. 3 and 4). In general, effects of benzodiazepines on respiration are described in terms of depression and shifts in carbondioxide response curves (14) and shifted hypoxic responses (2,28). In the present study, the $PaCO_2$ curves and the oxygen saturation curves were less affected. It seems that the depression of respiration provoked by opioids is enhanced by nonreactivity of the respiratory centers upon decreased oxygen in the blood. Administration of benzodiazepines in sedative doses induces a decrease in tidal volume and a compensatory increase in frequency, resulting in an unchanged minute volume (15), but here no sedative doses were used. We do not know whether other benzodiazepines would result in similar outcomes on respiration in rats and other species, including humans. Up to now, our observations indicate that a regimen of simultaneous administration of even low doses of opioids and a classical benzodiazepine should be used with caution in surgical patients, especially as during premedication.

In conclusion, the results presented here indicate that restraint in Bolman cages, although moderately stressful reflected by the increased corticosterone and prolactin levels, did not induce any restraint stress-induced analgesia as evidenced by the lack of antinociception in the vehicle-treated rats and the inability of a functional shift in the antinociceptive effects of low doses of morphine and sufentanil after pretreatment with an anxiolytic but nonsedative dose of the benzodiazepine chlordiazepoxide. Chlordiazepoxide, however, clearly worsened the opioid-induced shifts in blood gas values. It cannot be excluded from the present series of experiments that the relatively better blood gas values in saline-pretreated rats are the result from stimulation of the respiratory system by a moderate degree of stress evoked by residence in Bolman cages. Arterial blood gas sampling in freely moving rats, completed with measurement of respiratory variables such as tidal and minute volumes and frequency, could further elucidate this problem.

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