Pharmacology Biochemistry and Behavior, Vol. 44, pp. 633–641, 1993
Printed in the U.S.A.

0091-3057/93 $6.00 + .00

Combination of Buspirone and Other Drugs With β-CCE in Monkeys: Effects on Respiration and Behavior[1]

JOSEPH G. WETTSTEIN,[2] E. SUTTON TEEPLE AND W. H. MORSE

Harvard Medical School, Boston, MA 02115, and
New England Regional Primate Research Center, Southborough, MA 01772

Received 17 April 1992

WETTSTEIN, J. G., E. S. TEEPLE AND W. H. MORSE. *Combination of buspirone and other drugs with β-CCE in monkeys: Effects on respiration and behavior.* PHARMACOL BIOCHEM BEHAV 44(3) 633–641, 1993.—The respiratory and behavioral effects of the benzodiazepine receptor (BZR) inverse agonist ethyl-β-carboline-3-carboxylate (β-CCE) were determined alone and in combination with buspirone, lorazepam, flumazenil, and SR 95195 in rhesus monkeys. For the respiratory studies, one group of monkeys inhaled either air or 5% CO_2 mixed in air according to a fixed alternating schedule; respiratory frequency and minute volume were monitored. For the behavioral studies, another group of monkeys responded under a fixed-ratio (FR 30) schedule of food presentation. The respiratory stimulant effects of β-CCE in both air and 5% CO_2 were enhanced by prior treatment with the 5-hydroxytryptamine$_{1A}$ (5-HT$_{1A}$) partial agonist buspirone (0.03 and 0.3 mg/kg) and a weak BZR inverse agonist, SR 95195 (10.0 mg/kg). Coadministration of buspirone (0.1 and 0.3 mg/kg) also potentiated the rate-decreasing effects of β-CCE under the FR schedule. The BZR agonist lorazepam (3.0 mg/kg) and BZR antagonist flumazenil (1.0 mg/kg) attenuated the effects of β-CCE on respiratory frequency and minute volume particularly under the 5% CO_2 condition, and lorazepam (0.1 and 0.3 mg/kg) and flumazenil (0.1 and 0.3 mg/kg) attenuated the effects of β-CCE on FR responding. These latter results show that the respiratory and behavioral effects of β-CCE in rhesus monkeys are at least in part due to effects at BZRs. Moreover, the findings suggest either that coactivation of benzodiazepine and 5-HT$_{1A}$ sites lead to a greater than additive effect or that β-CCE and buspirone share a common mechanism of action that is unrelated to the receptor at which BZR inverse agonists act.

β-Carboline Buspirone Lorazepam Respiration Rhesus monkey Carbon dioxide
Anxiety Benzodiazepine β-CCE

DRUGS like ethyl-β-carboline-3-carboxylate (β-CCE) that have high affinity for benzodiazepine receptors (BZR) in brain tissue and pharmacological effects opposite those of BZR agonists are classified as BZR inverse agonists (5,15,21). In addition to having effects on their own, BZR inverse agonists can be used to antagonize the anxiolytic and other effects of BZR agonists such as lorazepam, chlordiazepoxide, and diazepam (16,19,25,29). In contrast to β-CCE and benzodiazepines, the azapirone derivative buspirone, widely indicated specifically for the treatment of anxiety, does not bind to BZRs. Buspirone is thought to exert its anxiolytic effects via an agonist action at 5-hydroxytryptamine$_{1A}$ (5-HT$_{1A}$) sites (11,20,31) and also has affinity for dopamine receptors (22).

The primary purpose of the present study was to examine the respiratory and behavioral effects of coadministration of β-CCE and buspirone in rhesus monkeys. Although much is known about both drugs, little is known about their joint effects. Individually, BZR inverse agonists and buspirone have respiratory stimulant properties in monkeys (30). It has been shown that β-CCE and another β-carboline, FG 7142, increased respiratory frequency without altering tidal volume in monkeys breathing air alone or 5% CO_2 mixed in air (5%

[1]This research was conducted at the New England Regional Primate Research Center and was supported by U.S. Public Health Service Grants DA 02658, MH 14275 and MH 07658 from the National Institute on Drug Abuse and the National Institute of Mental Health. Facilities and services were provided by the New England Regional Primate Research Center (U.S. Public Health Service, Division of Research Resources Grant RROO168) and by the Institut de Recherche Jouveinal. Animals used in this study were maintained in accordance with the guidelines of the Committee on Animals of the Harvard Medical School and the "Guide for the Care and Use of Laboratory Animals" (DHHS Publication No. (NIH) 85-23, revised 1985).

[2] Current address and address to which requests for reprints should be sent: Dr. Joseph G. Wettstein, Department of CNS Pharmacology, Institut de Recherche Jouveinal, 3-9 rue de la Loge, B.P. 100, 94265 Fresnes, France.

CO_2); the weak BZR inverse agonist CGS 8216 increased ventilation only in monkeys breathing air (30). Buspirone also increased respiratory frequency yet concurrently decreased tidal volume in monkeys breathing air or 5% CO_2 (30). In contrast to the similar respiratory effects of these drugs, BZR inverse agonists tend to only decrease schedule-controlled responding (1,3,10,29) whereas buspirone may increase or decrease responding in monkeys depending upon the schedule conditions (9,27,28). For comparison, the effects of β-CCE also were assessed in the presence of lorazepam, a BZR antagonist (flumazenil) (14), and a weak BZR inverse agonist (7-phenyl-3-methyl-1,2,4-triazolo-(4,3-b)-pyridazine, SR 95195) (4,23).

METHOD

Subjects

The respiration and behavioral experiments were conducted in separate groups of adult rhesus monkeys (*Macaca mulatta*) weighing 7.0–9.0 kg; each group had one female and two male monkeys. Monkeys lived in individual home cages between experimental sessions and were fed a diet of Purina Monkey Chow, fresh fruit, vegetables, and water. For experimental sessions, each monkey sat in a standard primate chair that was placed in a sound-attenuating chamber provided with an exhaust fan and white noise to mask extraneous sounds. Monkeys in the respiratory experiments had been studied previously under conditions similar to those described below and had received drugs (12,13,30); monkeys in the behavioral experiments were naive at the beginning of the study.

Respiratory Experiments

Apparatus. Specific details regarding the respiration apparatus have been described by Howell et al. (12). Briefly, each monkey was fitted with a rectangular Lexan helmet that served as a pressure displacement plethysmograph. A continuous flow (12 l/min) of 5% CO_2 mixed in air or air alone entered the helmet through a port in front of the monkey and was extracted through a port behind the monkey by a vacuum pump (Cole-Parmer Instrument Co., Model 7530-40). Flow meters monitored gas inflow and outflow. Changes in flow due to changes in respiration within the plethysmograph were measured by a pressure transducer (Statham, Los Angeles, CA, Model P23BC) connected to a polygraph (Grass Instruments, Inc., Quincy, MA, model 7) and a computer (IBM/PC). An integrator (Grass, Model 7PIOF) converted the flow signal to a volume measure. Respiratory frequency was determined directly and minute volume was determined by integration of the pressure-compensated plethysmograph signal. Tidal volume was calculated each minute as the quotient of minute volume and frequency.

Procedure and data analysis. Ventilation was monitored continuously with the polygraph and respiration parameters were averaged each minute throughout experimental sessions. The effects of drugs on respiration were determined in subjects exposed to 5% CO_2 mixed in air or air alone according to the following sequence. Initially, ventilation was recorded in isolated undisturbed subjects breathing air for about 30 min; monkeys were then injected with saline IM. Ten minutes after injection, the subject was exposed to 5% CO_2 for two 7-min periods each followed by 7 min of air (control period). Immediately after the second exposure to air, a second saline injection or a pretreatment injection (drug or vehicle) was given and the same sequence was repeated. Subsequently, test drugs or vehicle were administered IM every 24 min using the cumulative dosing procedure outlined below. Ten minutes after each dose of test drug, the monkey was exposed to 7 min of 5% CO_2 followed by 7 min of air. The last 4 min of each 7-min 5% CO_2 or air exposure were used to determine average values of frequency and minute volume for both pretreatment and test drugs. The effects of a dose of drug (or vehicle) on respiration were expressed as a percentage of the values obtained during the control period from the same session. Results were calculated for individual subjects and then averaged for the group. Mean effects of drugs were considered significant when the overall change in frequency or minute volume after a drug or drug combination differed by more than 2 SD from the mean control values of the group during vehicle control sessions. Group data for the respiratory experiments are represented because individual subjects responded similarly to individual drugs and combination of drugs.

Behavioral Experiments

Apparatus and schedule. A response lever was mounted on a transparent wall in front of the monkey. Each press of the lever produced an audible click within the chamber and was recorded as a response. Colored light bulbs could be illuminated to serve as visual stimuli. Food pellets (1 g; Noyes, L) could be delivered to a tray in the front wall. Monkeys responded under a fixed-ratio (FR) schedule of food presentation. In the presence of white light, 30 responses on the lever produced a pellet of food followed by a 30-s brief timeout. Sessions consisted of five sequential components, each of which had a duration of 10 min. FR components were separated by an extended 5-min timeout. Responding during timeouts had no scheduled consequence. Daily control sessions lasted approximately 75 min.

Data analysis. Rates of responding were calculated by dividing the total number of responses in a component by the total time the component was in effect. For each subject, rates of responding were relatively stable for the duration of the experiment. The day before each drug test session served as the respective control day; drug effects were expressed as a percent of control values determined on the previous day. Drug effects in individual monkeys were considered significant when the response rate after a drug or drug combination different from the mean control rate of that monkey by at least 2 SD. Data from individual monkeys are presented for the behavioral experiments because subjects frequently responded in a quantitatively different manner to individual drugs and combination of drugs.

Drugs and injection procedures

The drugs used were β-CCE (Research Biochemicals, Inc., Natick, MA), buspirone HCl (Mead-Johnson, Evansville, IN), lorazepam (Wyeth Laboratories, Philadelphia, PA), flumazenil (Hoffmann-La Roche, Nutley, NJ), and SR 95195 (Sanofi, Montpellier, France). Buspirone was dissolved in 0.9% sterile saline. Other drugs were dissolved in 95% ethanol, Emulphor EL-620P (GAF), and saline; propylene glycol (lorazepam) or hydrochloric acid (β-CCE; SR 95195) were added as required. Drugs were injected IM into calf or thigh muscles in volumes of approximately 0.12 ml/kg body weight. Control injections were similar volumes of the drug vehicles.

For the respiratory experiments, β-CCE was studied using a cumulative dosing procedure similar to those described earlier (12,30). After the control and pretreatment periods, incremental doses were injected 10 min prior to the start of each

5% CO_2 period. In a typical session, three or four cumulative doses of β-CCE were given; up to five different doses could be studied by determining overlapping cumulative dose-response functions in two separate sessions. In experiments with drug combinations, a single pretreatment dose of lorazepam, flumazenil, buspirone, or SR 95195 was injected IM 38 min prior to the first of two or three cumulative doses of β-CCE.

For behavioral experiments, β-CCE and buspirone were studied using a single-dose procedure. Drugs were administered IM 30 min before the start of experimental sessions. Each test session was preceded by a control session during which rates of responding were stable. In experiments with drug combinations, a single dose of lorazepam, flumazenil, or buspirone was injected IM immediately after injection of β-CCE. The order in which drugs were studied varied nonsystematically among monkeys.

RESULTS

Respiratory Experiments

Control respiration. Subjects maintained steady patterns of respiration during exposures to air and to 5% CO_2; mean frequency, tidal volume, and minute volume values are given in Table 1. Resting control values were constant over extended periods of time during and between daily sessions; variability of control measures was low (see SD in Table 1). Respiration rapidly increased during exposure to 5% CO_2 mixed in air and decreased back to prior levels during subsequent exposure to air alone; with each change, respiration reached a steady state within 3 min. Over 3-h vehicle-control sessions, the increases in respiration during reexposures to 5% CO_2 mixed in air were reproducible. Under these conditions, for example, the ventilatory effects of 5% CO_2 after 30 min or 2 h were not different from each other; ventilatory parameters during reexposures to air also remained constant (Table 1).

Effects of β-CCE alone and in combination with other drugs in monkeys breathing air. β-CCE (1.0–10.0 mg/kg) increased respiratory frequency and minute volume in monkeys breathing air (Fig. 1, solid boxes). Although not dose-dependent, increases in frequency and minute volume were observed in each subject, and the respiratory effects at the highest dose (10.0 mg/kg) differed from control values by more than two SD for the group of three monkeys. The effects of β-CCE on tidal volume were minimal; tidal volume was decreased to about 90% of control values at 1.0 mg/kg, and higher doses had less of an effect (data not shown).

The effects of single doses of buspirone, SR 95195, lorazepam, and flumazenil were determined during exposure to air immediately before administration of β-CCE. Buspirone (0.03 and 0.3 mg/kg) primarily increased frequency, lorazepam (3.0 mg/kg) and flumazenil (1.0 mg/kg) slightly decreased minute volume, and SR 95195 (10.0 mg/kg) had no effect on respiration in monkeys breathing air (Fig. 1, disconnected symbols). Buspirone, lorazepam, and flumazenil previously had been studied over a range of doses in monkeys breathing air: buspirone (0.03–1.0 mg/kg) increased frequency and minute volume to maximums of 156 and 128%, respectively, and decreased tidal volume to a minimum of 71%; lorazepam (0.1–10.0 mg/kg) decreased tidal and minute volume to minimums of 81 and 78%, respectively; and flumazenil (0.1–3.0 mg/kg) had few effects on ventilation (30). In the current study, SR 95195 was administered to monkeys in a cumulative manner over the dose range of 1.0–30.0 mg/kg and produced a modest dose-dependent increase in respiratory frequency and minute volume. Peak increases in frequency and minute volume, both to 126% of control values, occurred after 30.0 mgkg SR 95195; there were few effects on tidal volume at these doses in monkeys breathing air (data not shown).

Buspirone and SR 95195 potentiated the respiratory stimulant effects of β-CCE in monkeys breathing air (Fig. 1). In general, pretreatment with either 0.03 and 0.3 mg/kg buspirone or 10.0 mg/kg SR 95195 resulted in an upward shift in the β-CCE dose-response functions for frequency and minute volume. For both parameters, the effects with drug combinations were distinctly greater than those observed with β-CCE alone. For example, β-CCE alone (10.0 mg/kg) increased frequency and minute volume to about 130% of control values

TABLE 1

VENTILATION IN MONKEYS BREATHING AIR ALONE OR 5% CO_2 MIXED IN AIR

Measure	Control*	Time After the Control Period (min)†			
		30 min	60 min	90 min	120 min
Frequency					
Air	19 ± 3	98 ± 3%	98 ± 4%	102 ± 2%	100 ± 5%
5% CO_2	28 ± 3	97 ± 1%	98 ± 5%	101 ± 4%	97 ± 1%
Tidal volume					
Air	78 ± 6	101 ± 6%	100 ± 4%	100 ± 4%	99 ± 2%
5% CO_2	141 ± 14	101 ± 1%	101 ± 2%	101 ± 3%	101 ± 3%
Minute volume					
Air	1.4 ± 0.2	98 ± 7%	98 ± 7%	102 ± 3%	98 ± 5%
5% CO_2	3.9 ± 0.4	98 ± 1%	99 ± 5%	102 ± 5%	97 ± 3%

Control data were taken after one or two vehicle injections during the first hour of a test session. The remaining data were taken after sequential vehicle injections 30, 60, 90, and 120 min after the control period. Data are averages from 17 control sessions in 3 subjects.

*Control units (± 1 SD) are breaths/min for frequency, ml for tidal volume, and l/min for minute volume.

†Values at 30, 60, 90, and 120 min are means ± 1 SD expressed as a percent of the values represented in the "Control" column.

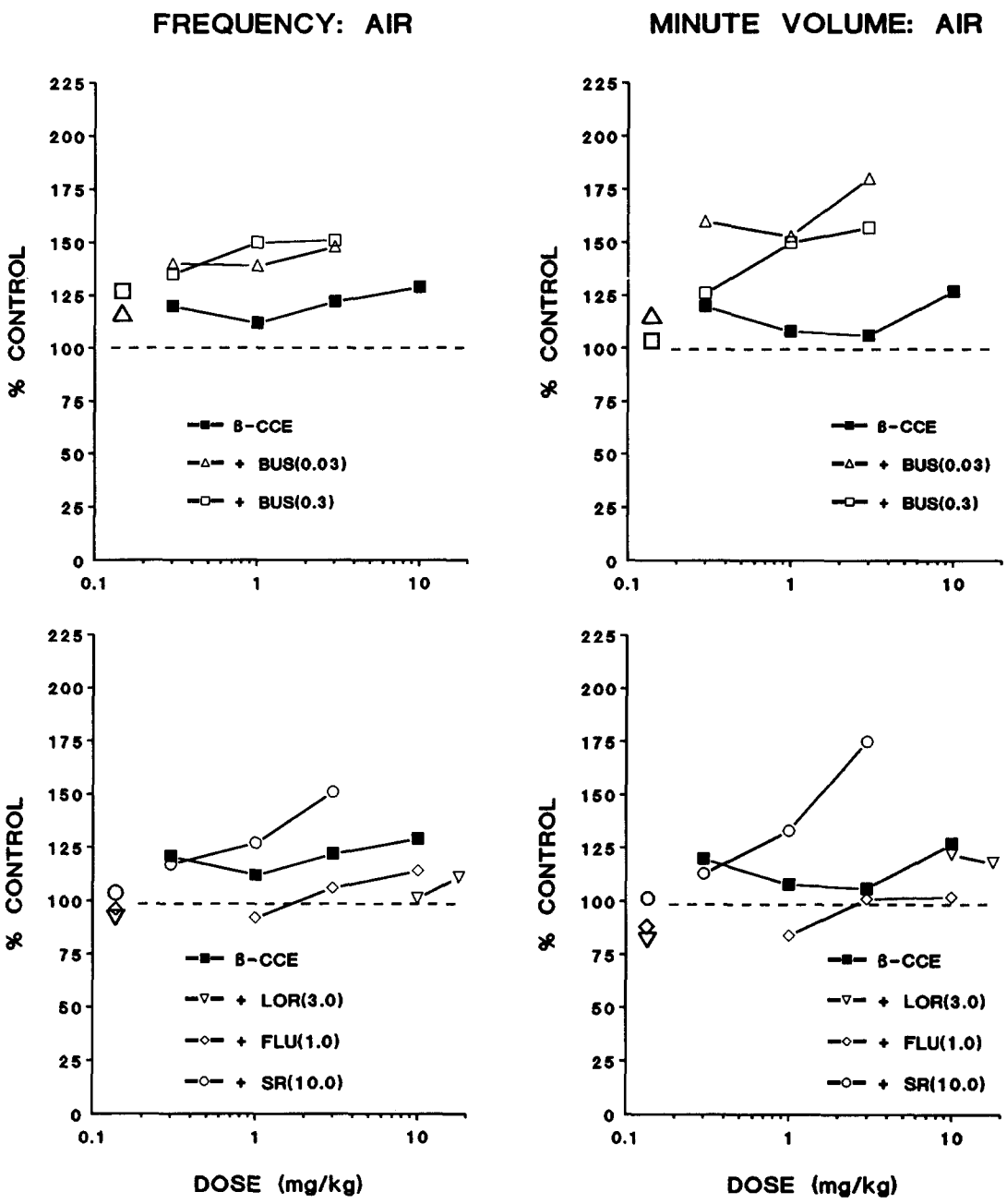

FIG. 1. Respiratory effects of β-CCE alone and in combination with buspirone, lorazepam, flumazenil and SR 95195 in monkeys breathing air. Abscissas: dose, log scale. Ordinates: respiratory frequency (left panels) or minute volume (right panels) expressed as a percent of control values. Data are means based on single determinations in each of three monkeys. The disconnected, unfilled symbols adjecent to the ordinates represent the effects of single administrations of 0.03 mg/kg lorazepam (inverted triangles), 1.0 mg/kg flumazenil (diamonds) and 10.0 mg/kg SR 95195 (circles); these data were obtained immediately prior to the subsequent cumulative dosing with β-CCE.

whereas after pretreatment with 0.03 mg/kg buspirone or 10.0 mg/kg SR 95195, a lower dose of β-CCE (3.0 mg/kg) elevated frequency to 150% and minute volume to over 170% of control values.

Flumazenil (1.0 mg/kg) and lorazepam (3.0 mg/kg) attenuated some of the respiratory effects of β-CCE in monkeys breathing air (Fig. 1, bottom panels). Flumazenil reduced the effects of β-CCE (10.0 mg/kg) on frequency from 129 to 114% and on minute volume from 127 to 102% of control values. With lorazepam, the effects were evident on frequency rather than on minute volume. Lorazepam reduced the effects of β-CCE (10.0 mg/kg) on frequency from 129 to 101% and minute volume was slightly altered from 127 to 123% of control values.

Effects of β-CCE alone and in combination with other drugs in monkeys breathing 5% CO_2 in air. β-CCE (0.3–10.0 mg/kg) produced a dose-dependent increase in frequency and minute volume in monkeys breathing 5% CO_2 (Fig. 2, solid boxes). β-CCE had no effect on tidal volume up to a dose of 10.0 mg/kg (data not shown). For the group of monkeys, the increases in respiratory frequency and minute volume differed from control values by more than 2 SD after administration of 3.0 and 10.0 mgkg β-CCE. Overall, the increases in the two respiratory measures were greater in monkeys breathing 5% CO_2 than breathing air (compare Figs. 1 and 2).

The effects of buspirone, SR 95195, lorazepam and flumazenil were determined during exposure to 5% CO_2 immediately before administration of β-CCE. The higher dose of buspirone (0.3 mg/kg) increased frequency and minute volume, SR 95195 (10.0 mg/kg) slightly increased frequency, lorazepam (3.0 mg/kg) decreased frequency and minute volume, and flumazenil (1.0 mg/kg) slightly decreased both parameters in monkeys breathing 5% CO_2 (Fig. 2, disconnected symbols). Earlier, buspirone, lorazepam, and flumazenil had been studied in monkeys breathing 5% CO_2: Buspirone (0.03–1.0 mg/kg) increased frequency and minute volume to maximums of

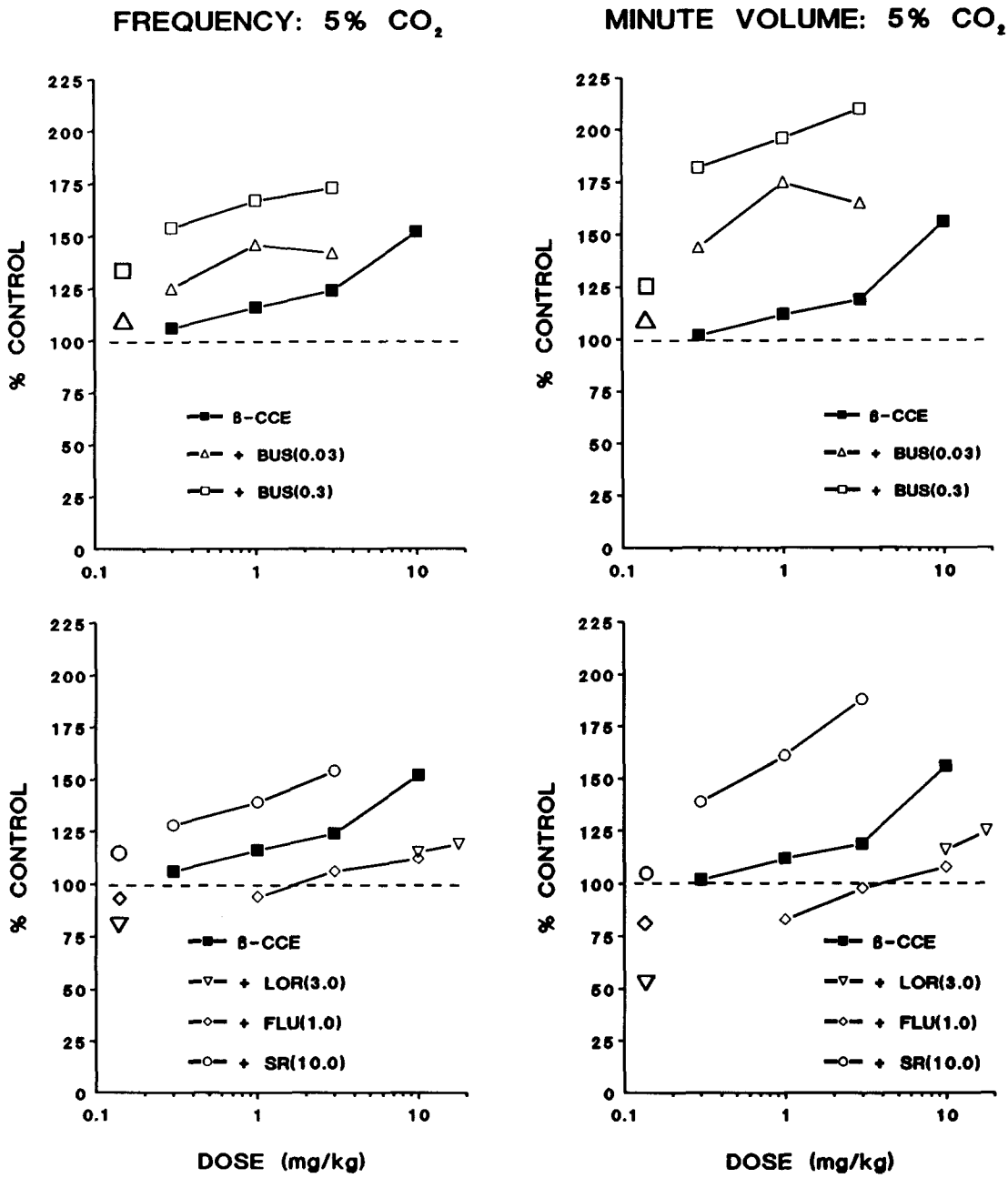

FIG. 2. Respiratory effects of β-CCE alone and in combination with buspirone, lorazepam, flumazenil and SR 95195 in monkeys breathing 5% CO_2. Other details are as in Fig. 1.

161 and 136%, respectively, and decreased tidal volume to a minimum of 78%; lorazepam (0.1-10.0 mg/kg) decreased tidal and minute volume to minimums of 59 and 50%, respectively; and flumazenil (0.1-3.0 mg/kg) slightly depressed tidal and minute volume (30). In the present experiments, SR 95195 was studied over a dose range of 1.0-30.0 mg/kg and was found to increase ventilation in air but not in 5% CO_2 (see above).

Buspirone and SR 95195 potentiated the respiratory stimulant effects of β-CCE in monkeys breathing 5% CO_2 (Fig. 2). Prior treatment with either buspirone (0.03 and 0.3 mg/kg) or SR 95195 (10.0 mg/kg) resulted in a leftward and upward shift in the β-CCE dose-response functions for frequency and minute volume. The effects with drug combinations clearly were greater than those observed with similar doses of β-CCE alone and were in particular evident on minute volume. Over

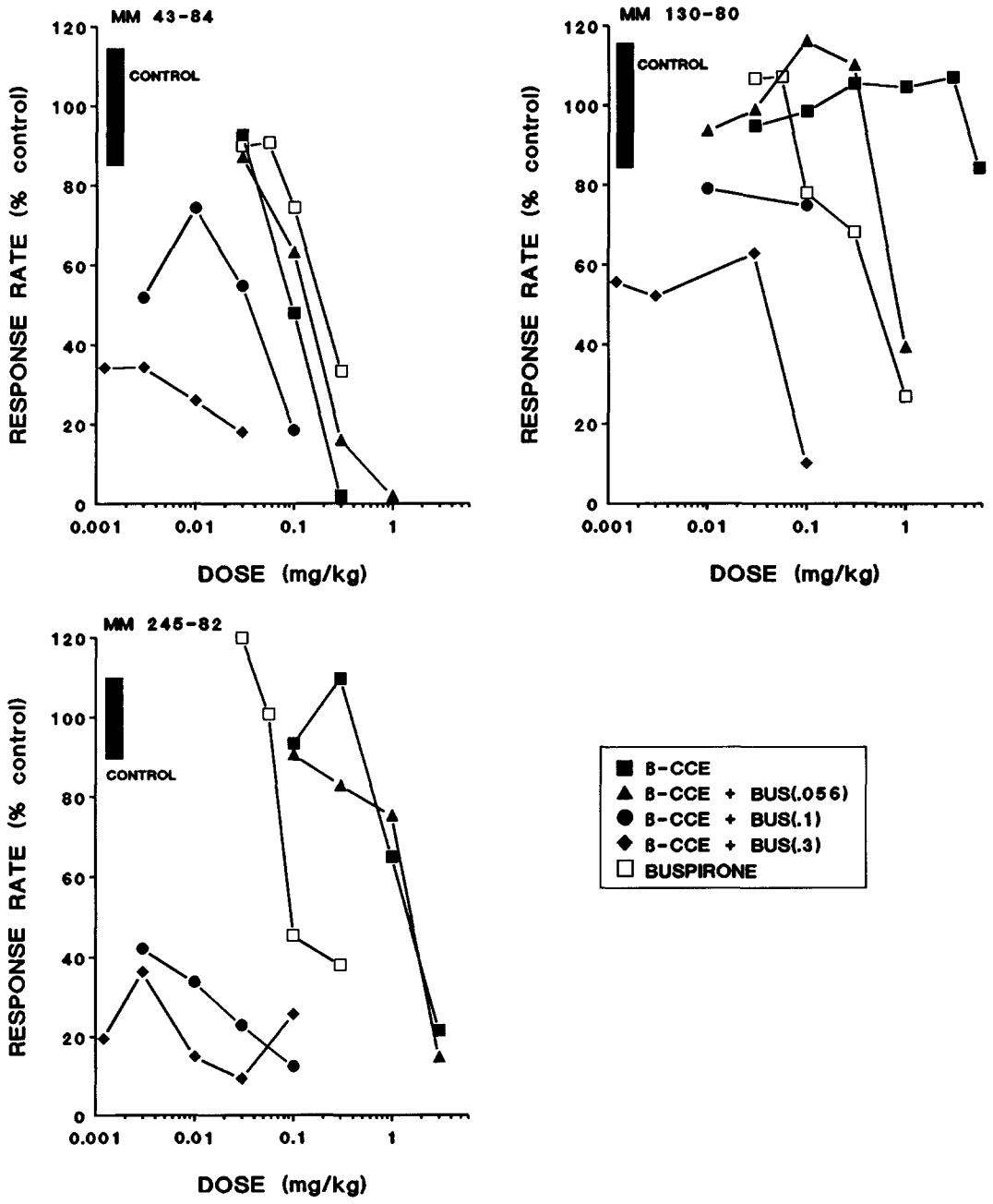

FIG. 3. Effects of β-CCE and buspirone (BUS), alone and in combination on FR responding in individual monkeys (MM 43-84, MM 130-80 and MM 245-82). Abscissas: dose, log scale. Ordinates: response rate (percent of control values). Data are means based on one or two determinations in each subject. Doses of buspirone coadministered with β-CCE are indicated in parentheses as mg/kg. The filled rectangles labelled "control" show the mean ± one S.D. response rate for that monkey.

the dose range of 1.0–10.0 mg/kg, β-CCE increased minute volume from 112–156% of control values, whereas after 0.3 mg/kg buspirone or 10.0 mg/kg SR 95195 minute volume was increased by a lower dose range of β-CCE (0.3–3.0 mg/kg) (Fig. 2).

Both lorazepam and flumazenil attenuated the respiratory effects of β-CCE in monkeys breathing 5% CO_2 (Fig. 2, bottom panels). The attenuation was observed over several doses of β-CCE and was evident for both frequency and minute volume measures. After pretreatment with lorazepam (3.0 mg/kg) or flumazenil (1.0 mg/kg), for example, the effects of 10.0 mgkg β-CCE on frequency were reduced from 152 to 114 or 113% and those on minute volume from 156 to 116 or 108% of control values, respectively.

Behavioral Experiments

Control performance. Control rates ±1 SD under the FR schedule for the three monkeys were 3.37 ± 0.55 (MM 130-80), 3.72 ± 0.45 (MM 245-82), and 5.02 ± 0.67 (MM 43-84) responses/s. Temporal patterns of FR responding were characteristic of those observed previously under FR schedules: responding occurred at a relatively constant rate after a slight pause at the beginning of each FR.

Effects of β-CCE alone and in combination with other drugs. β-CCE produced dose-dependent decreases in responding in two of three monkeys (Fig. 3, solid boxes). Significant decreases in response rate, those greater than 2 SD from control, were observed in monkey MM 43-84 after 0.1 and 0.3 mg/kg β-CCE and in monkey MM 245-82 after 1.0 and 3.0 mg/kg β-CCE. In the third subject (MM 130-80), the highest dose of β-CCE tested (5.6 mg/kg) decreased the rate to only 84% of the control value. Buspirone (0.056–0.3 mg/kg) decreased responding in a dose-dependent manner in all three monkeys (Fig. 3, open boxes).

The combination of increasing doses of buspirone with β-CCE resulted in leftward and downward shifts in the β-CCE dose-response function in each of the three subjects (Fig. 3). Doses of β-CCE equal to or much lower than those affecting FR responding markedly decreased the rate when administered together with 0.1 or 0.3 mg/kg buspirone in the individual monkeys. In contrast, both flumazenil and lorazepam, at doses that had no effect or decreased rate (data not shown), antagonized the rate-decreasing effects of β-CCE in the two monkeys studied (Fig. 4). In one monkey (MM 43-84), the antagonism was pronounced and dose dependent. In this subject, lorazepam and flumazenil, both at 0.1 mg/kg, produced modest rightward shifts in the β-CCE dose-response function whereas a higher dose of either drug (0.3 mg/kg) markedly shifted the β-CCE dose-response function to the right (Fig. 4, top). In the second monkey (MM 245-82), 0.3 mg/kg, but not 0.1 mg/kg, lorazepam and flumazenil attenuated the rate-decreasing effects of β-CCE (Fig. 4, bottom). These combinations of drugs were not studied in subject MM 130-80 because of both the observed resistance to the effects of β-CCE and the solubility limit with β-CCE.

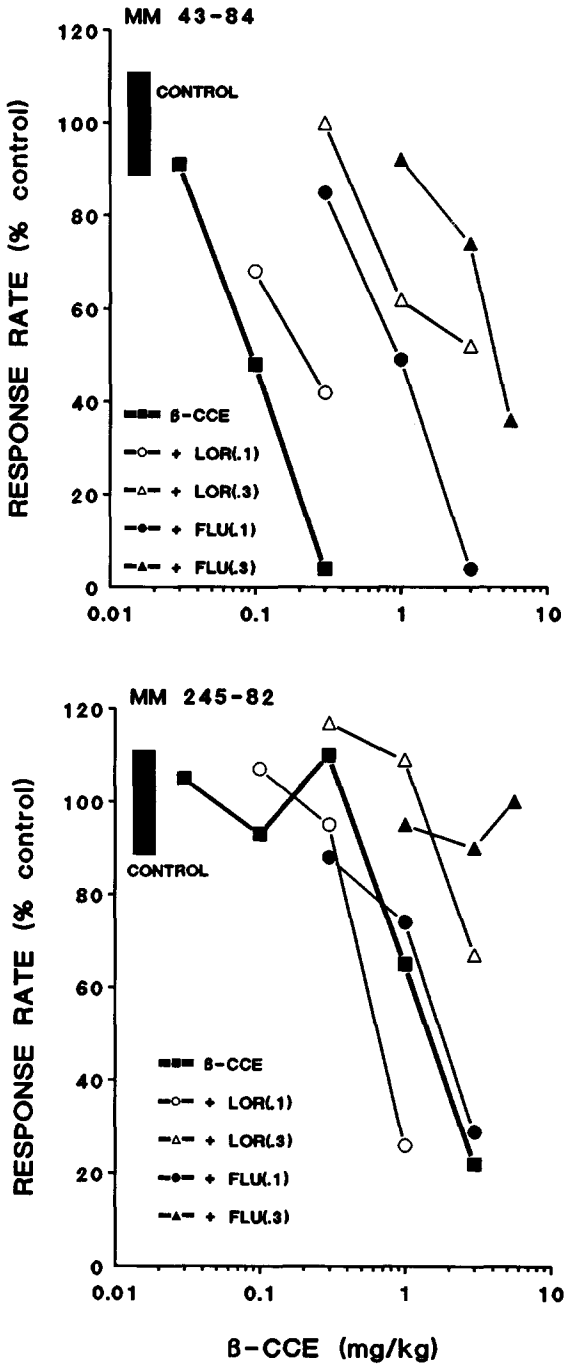

FIG. 4. Effects of β-CCE alone and after coadministration of lorazepam (LOR) or flumazenil (FLU) on FR responding in individual monkeys (MM 43-84 and MM 245-82). Other details are as in Fig. 3.

DISCUSSION

The respiratory stimulant and behavioral suppressant effects of β-CCE found in the present study can be considered characteristic for BZR inverse agonists. Other drugs from this class, including FG 7142 and CGS 8216, also have been found to increase minute volume in a dose-dependent manner in rhesus monkeys breathing air or 5% CO_2 (30). Moreover, under schedules of food or shock presentation β-carboline inverse agonists tend to only decrease responding in both rhesus and squirrel monkeys (1,3,10,29). β-CCE also has been reported to induced a series of endocrine, somatic, and overt behavioral effects in monkeys reflecting a state of anxiety or stress (7,18).

The results from the drug combination studies with flumazenil and lorazepam support the view that the effects of β-

CCE are primarily due to actions at BZR sites. As in the current study, results from other behavioral experiments involving monkeys and rodents have shown that the BZR antagonist flumazenil and BZR agonists consistently attenuate the response rate suppressant effects of different β-carboline inverse agonists (3,6,16,29). Under the FR schedule used in this experiment, the behavioral suppressant effects of β-CCE were antagonized by a dose of lorazepam known to also suppress response rate. The respiratory stimulant effects of β-CCE under both air and 5% CO_2 conditions in the present experiment also were attenuated by prior administration of flumazenil and lorazepam. The interpretation of a receptor-mediated antagonism between β-CCE and lorazepam is complicated, however, because the dose of lorazepam that attenuated the stimulatory effects of β-CCE depressed respiration when given alone. Nonetheless, these findings complement previous results showing that the stimulant effects of FG 7142 in monkeys breathing air or 5% CO_2 were reduced in a dose-dependent manner by the BZR agonists alprazolam and quazepam (30). In another study with rhesus monkeys, diazepam was found to not only attenuate the overt behavioral effects but also the cardiovascular and biochemical changes induced by β-CCE (7). It also was reported that the α-adrenergic agonist clonidine similarly affected the β-CCE response whereas a β-adrenergic agonist (propranolol), a serotonin antagonist (cyproheptadine), and a GABA-mimetic {4,5,6,7-tetrahydroisoxazolo[5,4-c]pyrindin-3-ol (THIP)} had mixed effects as antagonists of β-CCE (7).

The respiratory and behavioral effects of β-CCE were potentiated by buspirone in the present study. Despite buspirone having antianxiety effects in man (8), the present findings were not unexpected. Buspirone's antianxiety effects have been attributed at least in part to actions at 5-HT_{1A} receptors and involve mechanisms unrelated to those of BZRs (11,20,22, 31). Further, the behavioral profiles of buspirone and BZR agonists in both monkeys and rodents are noticeably different (17,28,32). 7,28,32).

In summary, this study has shown that the respiratory stimulant and behavioral suppressant effects of β-CCE in rhesus monkeys are likely due to effects at BZRs receptors. The potentiation of the effects of β-CCE by buspirone suggests that coactivation of benzodiazepine and 5-HT_{1A} receptors leads to a greater than additive effect. There is evidence indicating that BZR ligands alter the function of serotonergic systems and serotonergic agents alter functioning of the GABA/BZR complex [for review, see (24,26)]. Findings from this experiment alternatively suggest that the joint effects of β-CCE and buspirone in monkeys may be due to a common mechanism unrelated to the primary receptor site at which BZR inverse agonists act.

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