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Ventilatory Effects of Negative $GABA_A$ Modulators in Rhesus Monkeys

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GERAK, L. R., L. E. ESTUPINAN AND C. P. FRANCE. *Ventilatory effects of negative $GABA_A$ modulators in rhesus monkeys*. PHARMACOL BIOCHEM BEHAV **61**(4) 375–380, 1998.—This study examined changes in ventilation produced by negative γ-aminobutyric $acid_A$ ($GABA_A$) modulators in rhesus monkeys. The effects of Ro 15-4513, β-CCE and β-CCM were examined in four rhesus monkeys breathing air or 5% CO_2 in air. When monkeys breathed CO_2, minute volume (V_E) and frequency (f) increased, on average, to 158 and 140% of control (air), respectively. Ro 15-4513 did not modify ventilation in monkeys breathing either gas mixture; however, β-CCE and β-CCM increased V_E and f in monkeys breathing air to between 123 and 141% of control and had no effect on ventilation of 5% CO_2. Increased ventilation produced by the negative $GABA_A$ modulators appeared to be maximal, because ventilation was not further enhanced when the dose was increased three-fold. Each of the three negative $GABA_A$ modulators reversed the decreases in ventilation produced by diazepam, suggesting that these drugs are acting at benzodiazepine receptors; however, the increased ventilation produced by β-CCE and β-CCM might suggest that they have more negative efficacy than Ro 15-4513. These data extend previous findings by showing that some negative $GABA_A$ modulators (Ro 15-4513) do not alter ventilation and further indicate that changes in ventilation can be used to evaluate efficacy differences among $GABA_A$ modulators. © 1998 Elsevier Science Inc.

Negative $GABA_A$ modulators β-CCE β-CCM Ro 15-4513 Diazepam Ventilation Rhesus monkey

BENZODIAZEPINE receptors are located on the γ-aminobutyric $acid_A$ ($GABA_A$) receptor complex, and some drugs, including those acting at benzodiazepine receptors, can modulate the complex positively or negatively. For example, positive $GABA_A$ modulators potentiate the Cl^- flux produced by GABA and include benzodiazepine receptor agonists such as diazepam (13). In contrast, negative $GABA_A$ modulators inhibit the Cl^- flux produced by GABA and include benzodiazepine receptor inverse agonists such as DMCM [methyl-6,7-dimethoxyl-4-ethyl-β-carboline-3-carboxylate; (1)]. The bidirectional nature of drug action at benzodiazepine receptors is also evident for behavioral effects with positive $GABA_A$ modulators having anxiolytic and anticonvulsant effects (7) and negative $GABA_A$ modulators having anxiogenic (10) and convulsant effects (2). Although it is possible to measure the effects of either positive or negative $GABA_A$ modulators in a variety assays, few procedures can reliably measure the effects of both types of drugs when they are administered alone. Ventilation is one dependent variable that can be modified by both types of drugs; positive $GABA_A$ modulators decrease ventilation and negative $GABA_A$ modulators increase ventilation (14), thereby enabling studies of positive and negative $GABA_A$ modulators, either alone or in combination, in one procedure.

Another potential advantage of using ventilation to examine drugs that act at benzodiazepine receptors is that increased ventilation might be related to anxiety. For example, a number of physiologic changes, including increased ventilation, often accompany the subjective report of anxiety. In addition, some conditions that increase ventilation also produce anxiety. Negative $GABA_A$ modulators are anxiogenic in humans (10), and they can increase ventilation in rhesus monkeys (14). Increasing the concentration of CO_2 in air can also increase both ventilation (9) and anxiety (15). Healthy subjects report the effects of rebreathing CO_2 as similar to their life experiences of anxiety (15) and, in patients with panic disorder, increased concentrations of CO_2 can induce panic (12). In fact, increased concentrations of CO_2 have been proposed as a novel procedure for examining anxiety and for screening anxiolytic drugs (15).

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To determine whether increased ventilation in rhesus monkeys might be useful for studying positive or negative $GABA_A$ modulators and, therefore, predictive of anxiolytic and anxiogenic activity in humans, changes in ventilation were examined following administration of the negative $GABA_A$ modulators ethyl β-carboline-3-carboxylate (β-CCE), methyl β-carboline-3-carboxylate (β-CCM) and Ro 15-4513 in monkeys breathing air or 5% CO_2 in air. In addition, the ability of those drugs to reverse the ventilatory-depressant effects of diazepam was determined.

METHOD

Subjects

Three male and one female adult rhesus monkeys weighed between 6.0 and 10.0 kg during these experiments. These free-feeding weights were maintained with monkey chow (Teklad High Protein Monkey Diet) supplemented daily with fresh fruit and peanuts. Monkeys were housed individually on a 14 L:10 D schedule with unlimited access to water. In previous experiments, these monkeys received benzodiazepines, opioids, and other drugs.

These experiments were approved by the Institutional Animal Care and Use Committee, Louisiana State University Medical Center, New Orleans, and were conducted in accordance with the Guidelines and Principals of Laboratory Animal Care, National Research Council, Department of Health, Education and Welfare Publication (National Institutes of Health) 85-23, revised 1985.

Apparatus

During experimental sessions, monkeys were seated in restraint chairs and placed in sound-attenuating chambers. A helmet was placed over the head of the monkey with a series of plastic and latex neck dams used to prevent leakage of gas from the helmet. Gas (air or 5% CO_2 in air) was pumped into the helmet at a rate of 10 l/min and removed with a vacuum pump. Pressure changes in the helmet that occurred as the monkey breathed were measured with a transducer connected to a polygraph (Grass model 7); the pressure changes were transformed by an analog-to-digital converter and were expressed as f (inspirations/min) and V_T (ml/inspiration). V_E (ml/min) was the product of f and V_T.

The ventilation apparatus was calibrated daily prior to the experiment. A closed chamber was attached to the transducer, air tank, and vacuum pump; subsequently, a known amount of air was pumped in to the chamber 20 times per minute. The corresponding deflection of the polygraph pen was measured with the average value obtained over 3 min used as the calibration factor.

Procedure

The procedure for measuring ventilation has been described previously [e.g., (5)]. For dose–effect curve determinations, experimental sessions comprised several discrete 30-min cycles; during the first 23 min of each cycle, monkeys breathed air, and, during the last 7 min, monkeys breathed 5% CO_2 in air. Each session began with a 30-min control cycle during which drugs were not administered. Subsequently, increasing doses of drug were administered during the first min of each cycle so that the cumulative dose increased by 0.25 or 0.5 log U per cycle. Dosing continued up to the largest dose that could be safely administered (β-CCE and β-CCM) or to the limits of solubility (Ro 15-4513). One male monkey was particularly sensitive to the convulsant effects of the negative $GABA_A$ modulators and did not participate in these dose–effect determinations.

In addition to studying these three negative $GABA_A$ modulators alone, dose–effect curves were also determined in the presence of the smallest dose of diazepam (3.2 mg/kg) that produced a maximal decrease in ventilation (6). Prior to studying the effects of negative $GABA_A$ modulators in monkeys treated with diazepam, the duration of action of diazepam was determined by administering 3.2 mg/kg immediately after the control cycle; during five subsequent cycles, monkeys were handled but did not receive injections (i.e., "sham" injections). Once the time course of diazepam was known, the dose–effect curves for the negative $GABA_A$ modulators were determined. For those sessions, diazepam was administered during the cycle that immediately succeeded the control cycle. On subsequent cycles, increasing doses of a negative $GABA_A$ modulator were administered as described above.

Drugs

Ethyl beta-carboline-3-carboxylate (β-CCE), methyl beta-carboline-3-carboxylate (β-CCM) and diazepam (Research Biochemicals International, Natick, MA) were dissolved in a vehicle comprising 20% emulphor, 10% ethanol, and 70% water. Ro 15-4513 (ethyl 8-azido-6-dihydro-5-methyl-6-oxo-4H-imidazo[1,5-a]-[1,4]benzodiazepine-3-carboxylate; Research Biochemicals International) was dissolved in a vehicle comprising 40% propylene glycol, 50% saline, and 10% ethanol. Doses are expressed in terms of the forms listed above in mg/kg body weight and injections were administered SC in the back.

Data Analyses

For each monkey, values for V_E, V_T, or f obtained during the last 3 min of a component of a cycle (i.e., monkeys breathed air during the first 23-min component of the cycle and 5% CO_2 during the second 7-min component of the cycle) were averaged. These values were then expressed as a percentage of control values that were determined in monkeys breathing air during the first cycle of the session; the percentages were then averaged to determine the mean (±1 SEM) for the group. The time course for diazepam was determined twice in each of four monkeys, with the data averaged for individual monkeys prior to calculating the mean (±1 SEM) for the group. The dose–effect curves for the negative $GABA_A$ modulators in the absence of diazepam were determined once in only three monkeys to avoid toxicity in the fourth monkey, who was extremely sensitive to the convulsant effects of these drugs; the effects of the negative $GABA_A$ modulators studied in combination with diazepam were determined once in four monkeys. Because of the limited duration of action of diazepam, the entire dose–effect curves for β-CCE and Ro 15-4513 could not be determined in a single experimental session; consequently, these dose–effect curves were determined over several sessions with the largest dose of negative $GABA_A$ modulator used in one session being the smallest dose used in another session. Potency estimates for the negative $GABA_A$ modulators in reversing the ventilatory-depressant effects of diazepam were obtained by calculating the dose of the negative $GABA_A$ modulator required to increase V_E to 90% of control; ED_{90} values were selected for comparison because all three negative $GABA_A$ modulators increased V_E to >90% of control. Linear regression of the group means was used to determine these ED_{90} values. For control dose–effect curves, V_E,

V_T, and f determined in either air or CO_2 are plotted as a function of dose. For dose–effect curves determined in the presence of diazepam, only V_E in air is shown; qualitatively similar results were obtained in CO_2.

Although the negative $GABA_A$ modulators produced qualitatively similar effects among subjects, monkeys varied dramatically in sensitivity to these drugs; therefore, the group mean was not used to compare the ventilatory effects of the negative $GABA_A$ modulators to control. Instead, the raw data from individual monkeys were evaluated for statistical differences using one-way analysis of variance ($p \leq 0.05$) unless the test for normality or for equal variance failed, in which case a Kruskal–Wallis one-way analysis of variance on ranks was performed.

RESULTS

Individual and group mean control values for V_E, V_T, and f are shown in Table 1. The group mean values in monkeys breathing air were 1202.1 ml/min for V_E, 52.26 ml/inspiration for V_T, and 25.38 inspirations/min for f. When monkeys breathed 5% CO_2 in air, V_E increased to 158.0% of control, and f was increased to 139.8% of control breathing in air. V_T was not markedly altered when monkeys breathed CO_2.

β-CCM and β-CCE significantly enhanced ventilation in three and two monkeys, respectively. A dose of 0.1 mg/kg of β-CCM or 1.0 mg/kg of β-CCE increased the mean V_E to >127% of control (○ and □, upper panel, Fig. 1); larger doses did not further increase V_E. Ro 15-4513 did not alter the mean V_E up to the largest dose that could be administered (◇, upper panel, Fig. 1), although different effects were apparent in different monkeys. In subject GO, Ro 15-4513 significantly increased V_E, whereas V_E was unchanged in subject MA and significantly decreased in subject PR. Each of the three drugs decreased the mean V_T to 80% of control, although the effect was not dose related for either β-CCM or Ro 15-4513 (middle panel, Fig. 1). For each of the drugs, the decrease was significant in two of the three monkeys. In contrast, β-CCE and β-CCM dose dependently increased f with the largest increases occurring at the same doses that produced the maximum change in V_E (lower panel, Fig. 1); these increases in f were significant in two of the three monkeys. For Ro 15-4513, the decrease in the mean V_T was accompanied by a modest increase in f to 120% of control, resulting in no net change in the mean V_E. The increase in f was significant in one monkey.

Increasing the concentration of CO_2 to 5% increased V_E, on average, to 165% of control (points above S, upper panel, Fig. 2) and this change in V_E appeared to be primarily due to increases in f (points above S, middle and lower panels, Fig. 2). In monkeys breathing CO_2, the mean V_E was not markedly changed by the negative $GABA_A$ modulators (upper panel, Fig. 2). Ventilation in CO_2 was significantly increased by each of the negative $GABA_A$ modulators in one monkey, significantly decreased by β-CCM and β-CCE in the other two monkeys and significantly decreased by Ro 15-4513 in one monkey. Similarly, neither the mean V_T nor f were dramatically altered by any of the negative $GABA_A$ modulators, although f was significantly increased in all monkeys that received β-CCE (middle and lower panels, Fig. 2).

A dose of 3.2 mg/kg of diazepam decreased the mean V_E to <75% of control (points above D, Fig. 3) and ventilation remained significantly decreased in all monkeys for a minimum of 90 min following diazepam administration (data not shown). Each of the three negative $GABA_A$ modulators tended to reverse this decrease in V_E (Fig. 3); this effect was significant in three monkeys that received β-CCE, two monkeys that received β-CCM and four monkeys that received Ro 15-4513. Moreover, diazepam prevented the increased ventilation produced by β-CCE (left panel, Fig. 3) and β-CCM (center panel, Fig. 3), and diazepam also prevented toxicity (data not shown) so that a three-fold larger dose of β-CCE could be safely administered in the presence of diazepam compared to control conditions. When administered in combination with diazepam, Ro 15-4513 slightly increased V_E to 110% of control at a dose (1.0 mg/kg) that had no effect on ventilation when administered alone (right panel). Based on the ED_{90} values, the rank order potency of the three negative $GABA_A$ modulators was Ro 15-4513 (ED_{90} = 0.047 mg/kg) = β-CCM (ED_{90} = 0.077 mg/kg) < β-CCE (ED_{90} = 1.17 mg/kg).

DISCUSSION

In the current study, the negative $GABA_A$ modulators β-CCE and β-CCM (2,11) increased ventilation in rhesus

TABLE 1

V_E (ML/MIN), V_T (ML/INSPIRATION) AND f (INSPIRATIONS/MIN) DETERMINED UNDER CONTROL (NO DRUG) CONDITIONS

	Air			CO_2		
Subject	V_E	V_T	f	V_E	V_T	f
GO	1342.4 ± 94.7*	79.76 ± 5.88	16.90 ± 0.35	2317.4 ± 95.9 (172.6)†	84.67 ± 4.67 (106.2)	27.60 ± 0.74 (163.3)
MA	1597.8 ± 115.0	38.18 ± 2.45	42.07 ± 1.58	2010.7 ± 129.1 (125.8)	45.60 ± 2.97 (119.4)	44.20 ± 0.60 (105.1)
PR	927.2 ± 88.7	50.76 ± 6.08	19.27 ± 1.12	1612.2 ± 121.9 (173.9)	46.86 ± 3.83 (92.3)	34.64 ± 0.93 (179.8)
ER	940.9 ± 76.1	40.34 ± 2.85	23.27 ± 0.69	1656.9 ± 125.5 (176.1)	46.67 ± 3.09 (115.7)	35.43 ± 0.85 (152.3)
Mean	1202.1 ± 163.3	52.26 ± 9.57	25.38 ± 5.72	1899.3 ± 165.4 (158.0)	55.95 ± 9.58 (107.1)	35.47 ± 3.40 (139.8)

*Mean (±SEM) of 10 control cycles; values for individual cycles are the average of values obtained during minutes 21–23 of exposure to air or minutes 5–7 of exposure to 5% CO_2.

†Value obtained during exposure to 5% CO_2 expressed as a percentage of value determined during exposure to air.

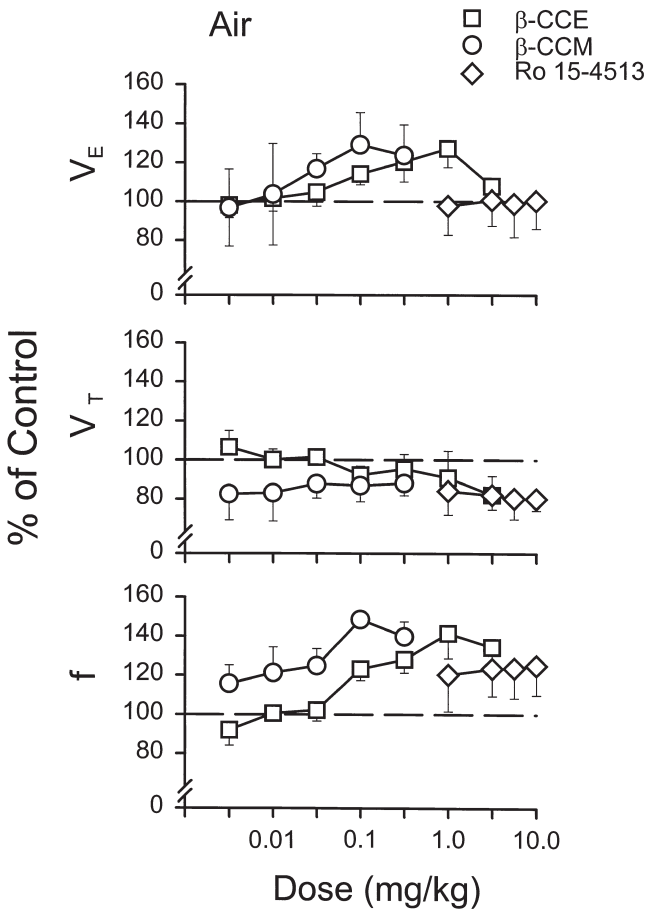

FIG. 1. Effects of negative $GABA_A$ modulators on ventilation in monkeys breathing air. Each dose–effect curve was determined once in each of three monkeys. Abscissae: dose in mg/kg body weight. Ordinates: V_E, V_T, or f expressed as a percentage of control (no drug) values in monkeys breathing air (mean ± 1 SEM).

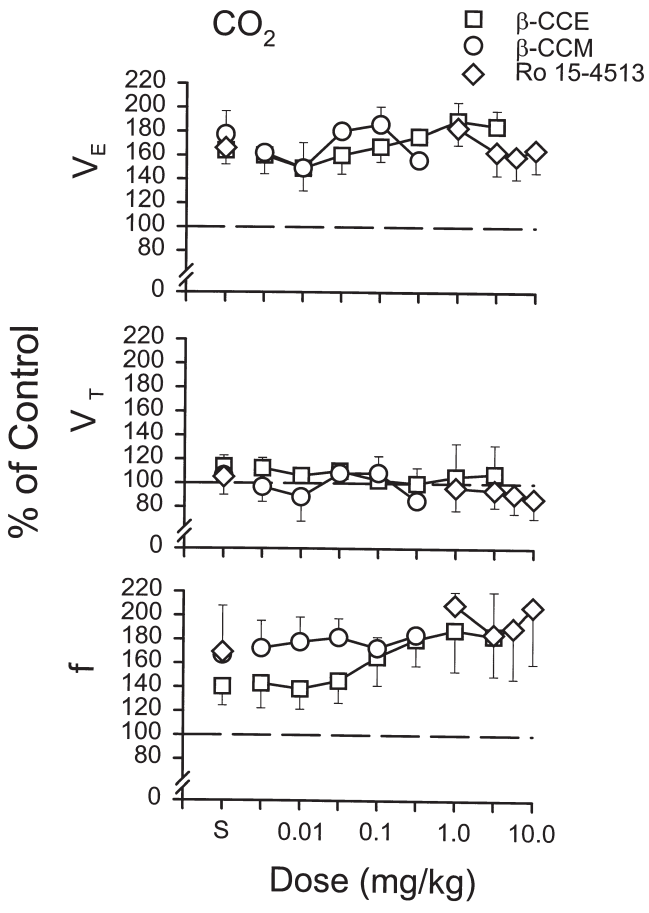

FIG. 2. Effects of negative $GABA_A$ modulators on ventilation in monkeys breathing 5% CO_2 in air. See Fig. 1 for other details.

monkeys breathing air. These data are consistent with another study in which β-CCE produced similar increases in ventilation in rhesus monkeys (14). Moreover, β-CCE and β-CCM reversed the ventilatory-depressant effects of diazepam, and the doses required to reverse the effects of diazepam were not markedly different from the doses required to increase ventilation when the negative $GABA_A$ modulators were administered alone, suggesting that both effects were mediated by the same receptor. In contrast, the negative $GABA_A$ modulator Ro 15-4513 (4) did not modify ventilation, although Ro 15-4513 clearly has effects at benzodiazepine receptors because a 30-fold smaller dose reversed the ventilatory-depressant effects of diazepam. The inability of Ro 15-4513 to increase ventilation when administered alone suggests that β-CCE and β-CCM have more negative efficacy than Ro 15-4513; if this supposition is true, then Ro 15-4513 should antagonize the ventilatory-stimulating effects of β-CCE and β-CCM. This notion is further supported by other studies showing that Ro 15-4513 has proconflict and proconvulsant effects that are antagonized by flumazenil, indicating that Ro 15-4513 is a negative $GABA_A$ modulator, and studies showing that Ro 15-4513 antagonizes the convulsant effects of DMCM, another negative $GABA_A$ modulator, indicating that DMCM has more negative efficacy than Ro 15-4513 (4). The data from the current study extend previous findings by showing that only some negative $GABA_A$ modulators can increase ventilation and suggest that this procedure can be used to evaluate variations in efficacy at benzodiazepine receptors. Specifically, the sensitivity of this procedure for detecting differences in efficacy is not limited to differentiating broadly between positive and negative $GABA_A$ modulators because, under these conditions, the procedure appears to be appropriate for detecting efficacy differences among negative $GABA_A$ modulators.

In rhesus monkeys, ventilation can also be enhanced by increasing the concentration of CO_2 in air, and humans have reported that the effects of increased CO_2 are similar to life experiences of anxiety (15). Negative $GABA_A$ modulators also can produce anxiety in humans (10); together, these data suggest that, under some conditions, increased ventilation might be related to anxiety and that ventilation in monkeys might provide a useful method for examining anxiety-related phenomena. In the current study, the magnitude of the ventilatory effects produced by 5% CO_2 was greater than the increases produced by the negative $GABA_A$ modulators. For example, V_E was increased to 158% of control when monkeys breathed 5% CO_2 in air, whereas V_E was increased maximally to 129% of control following administration of either β-CCE or β-CCM. Moreover, the ventilatory-stimulating effects of

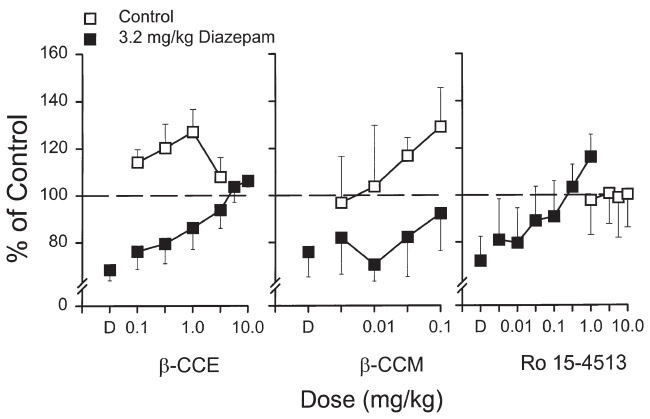

FIG. 3. Interactions between diazepam and each of the three negative $GABA_A$ modulators. Points above D indicate the ventilatory-depressant effects of a dose of 3.2 mg/kg of diazepam administered at the beginning of the first drug cycle. Increasing doses of a negative $GABA_A$ modulator were administered during subsequent cycles. The control dose–effect curves are identical to those shown in Fig. 1. The dose–effect curves for the negative $GABA_A$ modulators studied in combination with diazepam were determined once in each of four monkeys. Abscissae: dose in mg/kg body weight. Ordinates: V_E expressed as a percentage of control (no drug) values in monkeys breathing air.

these drugs and those of 5% CO_2 did not appear to be additive; that ventilation in these monkeys might have increased to some physiologic limit does not appear to account for a lack of interaction between the two stimuli because other studies have demonstrated that ventilation in rhesus monkeys can be two- to three-fold greater than the maximum increases obtained in the current study (3,8,14). This lack of additivity might suggest that different mechanisms mediate the ventilatory-increasing effects of negative $GABA_A$ modulators and 5% CO_2; however, positive $GABA_A$ modulators attenuate the ventilatory-enhancing effects of 5% CO_2 (6,14), indicating that the mechanisms are not completely independent. Thus, despite the fact that both negative $GABA_A$ modulators and increased CO_2 can produce subjective reports of anxiety (10,14) and can increase ventilation, it is not clear from the current studies whether increased ventilation can be used to predict increased anxiety.

In the current study, increased V_E appeared to be due to increased f with V_T either unchanged or decreased, regardless of whether the ventilatory-stimulant effects were produced by negative $GABA_A$ modulators or by 5% CO_2. These data are consistent with another study in rhesus monkeys in which the negative $GABA_A$ modulators β-CCE and FG 7142 produced comparable increases in V_E by increasing f (14). In contrast, there are qualitative differences among studies in the effects of positive $GABA_A$ modulators on the ventilatory components that contribute to V_E. In a previous study conducted in this laboratory using the same equipment and experimental subjects, the decreases in V_E produced by positive $GABA_A$ modulators were due to a decrease in f (6); thus, under the conditions used in the current study, changes in ventilation consistently resulted from changes in f. However, under other conditions (14), decreased ventilation produced by positive $GABA_A$ modulators was due to a decrease in V_T with no change in f, indicating that the ventilatory components of V_E can be modified differentially depending on whether ventilation was increased or decreased. Despite these differences among studies, some positive $GABA_A$ modulators (e.g., benzodiazepine receptor agonists) consistently decrease ventilation and some negative $GABA_A$ modulators (e.g., benzodiazepine receptor inverse agonists) consistently increase ventilation in rhesus monkeys, with the magnitude of the effects being comparable among studies. Moreover, flumazenil antagonizes the ventilatory-depressant effects of benzodiazepine agonists (6,14) and benzodiazepine agonists attenuate the ventilatory-stimulant effects of inverse agonists [current study; (14)], suggesting that differences in the effects of positive $GABA_A$ modulators on the components of ventilation (V_T and f) would not dramatically alter conclusions of studies examining interactions among drugs that vary in efficacy at benzodiazepine receptors.

Drugs that act at benzodiazepine receptors can vary widely in efficacy, and there are many procedures that can detect drugs that are either positive or negative $GABA_A$ modulators. In contrast, there are few procedures in which the effects of both positive and negative $GABA_A$ modulators, administered alone, can be examined reliably and safely. Positive $GABA_A$ modulators, such as diazepam, decrease ventilation in rhesus monkeys and negative $GABA_A$ modulators, such as β-CCE, increase ventilation. Moreover, other drugs that are known to produce effects through benzodiazepine receptors, such as Ro 15-4513, do not modify ventilation. Thus, this procedure might be useful not only in assessing differences in efficacy among drugs that act at benzodiazepine receptors, based on whether they increase, decrease, or have no effect on ventilation, but also in understanding interactions among drugs that vary in efficacy at benzodiazepine receptors.

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