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Differential Actions of RO 15-1788 and Diazepam on Poikilothermia, Motor Impairment and Sleep Produced by Ethanol

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PAEZ, X. AND R. D. MYERS. *Differential actions of RO 15-1788 and diazepam on poikilothermia, motor impairment and sleep produced by ethanol.* PHARMACOL BIOCHEM BEHAV **36**(4) 915–922, 1990.—In adult male Sprague-Dawley rats kept at an ambient temperature of 23–25°C, ethanol was injected intraperitoneally in a dose of 4.0 g/kg to produce a clear-cut impairment of autonomic and motorial functions. Following the injection of ethanol, motor coordination, measured on a rotorod, behavioral sleep, righting reflex and colonic temperature were monitored at predetermined intervals for 5.0–7.0 hr. In the first experiment, either 1.0 mg/kg RO 15-1788 (flumazenil), a benzodiazepine (BZ) receptor antagonist, or 1.0–5.0 mg/kg diazepam, a classical benzodiazepine receptor agonist, were injected intraperitoneally either alone or concurrently with ethanol's administration. In the second study, either RO 15-1788 (1.0 or 2.0 mg/kg) or diazepam (1.0 or 5.0 mg/kg) was injected at the nadir of the fall of body temperature induced by ethanol. Although RO 15-1788 alone failed to affect the rats' temperature, it did not prevent the characteristic ethanol-induced hypothermia but rather potentiated it in a dose-dependent manner. Further, this BZ receptor antagonist exacerbated motor incoordination and other behavioral effects when given either simultaneously with ethanol or at the nadir in the animals' core temperature. Although diazepam evoked a dose-dependant hypothermia, it did not enhance ethanol-induced hypothermia when both drugs were administered simultaneously. However, diazepam augmented motor incoordination and other effects and served to delay their recovery. When given to the rats at the nadir of ethanol hypothermia, diazepam did not potentiate ethanol's thermolysis but retarded the recovery from hypothermia; it caused also a dose-dependent delay in the recovery of motor coordination and other responses. These results show that diazepam and RO 15-1788 in this dose range do not act in an opposite manner as receptor agonist and antagonist, respectively, in terms of their actions on ethanol induced autonomic and motor impairment. Nevertheless, since the BZ agonist and antagonist exhibit differential functional effects, it thus is envisaged that diazepam and ethanol could share a similar substrate and site of action in the brain to produce hypothermia. On the other hand, RO 15-1788 could act at the level of signal transduction in brainstem neurons to potentiate the effects of ethanol. However, since both drugs augment incoordination, a common central mechanism of action is implied for motor incoordination which is distinguishable from the autonomic incapacitation induced by ethanol.

Ethanol Brain Temperature Thermoregulation RO 15-1788 Flumazenil Benzodiazepine receptors
Alcohol Sleep Righting reflex Motor coordination Poikilothermia

NUMEROUS compounds including ethanol act centrally on different neuronal systems in the brain to precipitate an incapacitation of both autonomic and motor functions (19, 28, 29). Among the conspicuous pathophysiological effects of ethanol is its anesthetic-like action on the hypothalamic system for the control of body temperature.

Initially, a cellular Ca^{2+} ion mechanism involving the temperature set-point was implicated in the poikilothermic action of ethanol. When the calcium chelator, EGTA, was infused centrally the thermolytic effect of this and other anesthetic drugs was reversed (24,26), thereby reinstating the impaired set-point temperature (27). Hypothalamic receptors for 5-HT and catecholamines in the anterior hypothalamic/preoptic area (AH/POA) involved in the thermoregulatory mechanism (25) were not implicated in ethanol thermolysis because pharmacological antagonists of monoamine receptors injected centrally failed to alter ethanol-induced changes in core temperature (26). However, studies using local in vivo perfusion revealed that both 5-HT and NE are released reciprocally within the AH/POA during temperature changes produced by ethanol (17,18). Further, as based on α_2-receptor antagonist

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TABLE 1

MEAN ± S.E. MAGNITUDE OF CHANGE FROM BASELINE IN CORE TEMPERATURE (T_b) IN °C OF RATS AT 15 MIN AND 1.0 HR AFTER INTRAPERITONEAL ETOH, INTRAPERITONEAL RO 15-1788 (DOSE IN mg/kg) OR BOTH

Treatment	15 Min	1.0 Hr	Maximum	Hr to Nadir
ETOH (N=6)	−0.8 ± 0.1	−1.6 ± 0.3	−2.1 ± 0.2	1.3 ± 0.2
RO 15-1788 1.0 mg (N=5)	+0.2 ± 0.1	−0.1 ± 0.2	−0.3 ± 0.2	1.7 ± 0.1
RO 15-1788 1.0 mg + ETOH (N=5)	−0.7 ± 0.1	−1.6 ± 0.2	−2.8 ± 0.2	2.3 ± 0.2

Maximum decline in T_b and hours to nadir are shown.

studies, adrenoreceptors in the periphery could also be involved in the poikilothermia produced by ethanol (12).

Central mechanisms involving the GABA-benzodiazepine (BZ) receptor complex also have been implicated in the control of core temperature and other physiological processes (19,34). For example, diazepam in 2.5–10 mg/kg doses potentiates hypothermia of rats exposed prenatally to ethanol, suggesting a long-term action on the thermoregulatory system of ethanol exposure in utero (36). Further, Bonetti *et al.* (6) found that RO 15-1788, an imidodiazepine which specifically displaces [^{3}H]-BZ from binding sites, acts to reverse the antagonistic effect of the partial BZ receptor inverse agonist, RO 15-4513, on the CNS depressant effect induced by ethanol. Although RO 15-1788 may have little or no effect on temperature alone, it does antagonize hypothermia caused by benzodiazepines on an inverse agonist of BZ receptors (37). Other evidence of the BZ receptor antagonist interaction with ethanol is the finding that RO 15-1788 potentiates the effect of ethanol in the animal as it accepts more punishing shock (2,3). On the other hand, the behavioral intoxication following ethanol and its antagonism by RO 15-4513 are not reversed by RO 15-1788 (15). Although RO 15-1788 potentiates the incoordination induced by ethanol, it is reported to exert little or no antagonistic effect on the hypothermia or sedative effects induced by ethanol (5,9).

In view of these findings, the present study was undertaken to compare the effects of the classical BZ receptor agonist, diazepam, with the classical antagonist, RO 15-1788, on autonomic and other functional impairments induced by ethanol at room temperature. Efficacious doses of both drugs were administered intraperitoneally to ascertain their effect on the core temperature of the rat alone and after an intoxicating dose of ethanol was given. Measures were obtained also of motor coordination, behavioral sleep and reflexes in relation to the administration of the drugs.

METHOD

Male rats (N=22) of the Sprague-Dawley strain, weighing from 250 to 350 g, were housed individually in wire mesh cages. Purina rat food and water were always available ad lib. During an experiment, each rat was placed in a 45×23×21 cm plastic chamber in a Laboratory room kept at an ambient temperature of 23–25°C.

Preparation of Drugs

A v/v solution of 20% ethanol was prepared with 95% reagent grade ethanol in glass distilled water so that in all experiments the dose of ethanol used was always 4.0 g/kg given intraperitoneally. This dose was selected on the basis of earlier studies (23,28).

RO 15-1788 (Hoffmann-La Roche) was prepared in warm sterile distilled water adjusted to a pH of 5.0 with acetic acid in doses of 1.0 and 2.0 mg/kg. Diazepam (Elkins-Sinn Inc.) in doses of 1.0 and 5.0 mg/kg was diluted in a sterilized vehicle containing 40% propylene glycol, 10% ethyl ethanol and 50% distilled water. Both compounds were given by the intraperitoneal route, in a concentration of 1.0 ml/kg.

Test Procedures

Before an experiment was begun at 0700–0730 hr, a YSI No. 401 thermistor probe was inserted into the colon of the rat so that body temperature (T_b) could be monitored every 15 min for the first 3 hr and every hr thereafter for 3–7 hr. At each of these intervals, simultaneous measures were taken of 1) behavioral sleep, as reflected by eye closure and inactivity; 2) arousal which was scored at the time when the rat opened its eyes and began turning its head in orienting toward a sensory stimulus (11); 3) loss of righting reflex, which was assessed according to the criterion that the rat failed to right itself to the ventral position within 10 sec after its placement on the dorsal surface of the body (22); and 4)

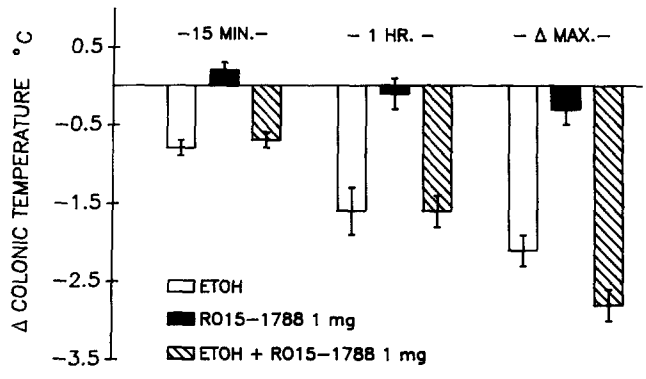

FIG. 1. Mean ± S.E. changes in °C of colonic temperature at 15 min, 1.0 hr as well as maximum shift inT_b under three conditions: ethanol (ETOH) administered intraperitoneally alone in dose of 4.0 g/kg; RO 15-1788 injected intraperitoneally alone in a dose of 1.0 mg/kg; and ethanol plus RO 15-1788 in the same doses administered simultaneously.

TABLE 2

PERCENT OF RATS DISPLAYING BEHAVIORAL SLEEP, AREFLEXIA AND INCOORDINATION AT 15 MIN AND 1.0 HR (TIME COURSE) AFTER INTRAPERITONEAL ETOH (N = 6), INTRAPERITONEAL RO 15-1788 (N = 5) (DOSE IN mg/kg) OR BOTH (N = 5)

	Sleep and Areflexia Time Course		Arousal and Reflex Intact	
Treatment	15 Min	1.0 Hr	Onset	Complete
ETOH	100%	100%	40% 4.5 hr	100% 6.0 hr
RO 15-1788 1.0 mg	0%	0%	No effect	No effect
RO 15-1788 1.0 mg + ETOH	100%	100%	60% 5.5 hr	100% 6.0 hr
	Incoordination Time Course		Coordination	
	15 Min	1.0 Hr	Onset*	Complete
ETOH	100%	100%	60% 6.0 hr	100% 7.0 hr
RO 15-1788 1.0 mg	0%	0%	No effect	No effect
RO 15-1788 1.0 mg + ETOH	100%	100%	0% 6.0 hr	0% 7.0 hr

*Walking on rotorod but not to 3 min criterion.

motor coordination, as assessed on a variable speed rotorod (Model 7700 treadmill for rats, Ugo Basile, Varese, Italy) set at a constantly rotating speed of 12.5 RPM (10) on which each rat had been trained to remain. The criterion for incoordination was the inability of the rat to remain on the rotorod for a period of 3.0 min. The order of recording data at the specific time intervals was as follows: body temperature, sleep-arousal, righting reflex and motor coordination.

Experimental Design

Two experimental designs were used to determine whether the effects of ethanol on body temperature, sleep and motor coordination could be attenuated or otherwise altered. In the first, either a 1.0 mg/kg dose of RO 15-1788 or 1.0 or 5.0 mg/kg diazepam was administered simultaneously with ethanol. In addition, two control conditions were also employed in which either ethanol was administered alone or RO 15-1788 and diazepam were given independently of ethanol. Efficacious doses of the compounds were selected on the basis of earlier studies.

In the second design, two doses of both RO 15-1788 (1.0 or 2.0 mg/kg) and diazepam (1.0 or 5.0 mg/kg) were administered at the point in time when the effects of ethanol on body temperature, sleep, righting reflex and motor coordination reached their maximum. Body temperature and other behavioral responses were recorded every 15 min for up to 3 hr under these test conditions. An interval of at least 4–5 days elapsed between each of the tests in which ethanol was administered in order to avoid the potential development of tolerance to the drug (29).

The data were analyzed statistically by computer using the Stat-Mate software program. One-way analyses of variance were performed followed by Neuman-Keuls analyses when appropriate. A p-value of <0.05 was considered to be statistically significant.

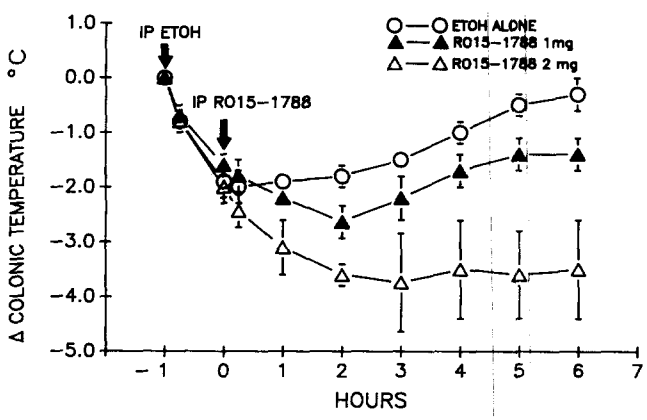

FIG. 2. Mean ± S.E. deviation in °C of colonic temperature over time in hr of rats given 4.0 g/kg ETOH intraperitoneally (ETOH ALONE) at −1 hour, or followed by RO 15-1788 injected intraperitoneally in doses of 1.0 or 2.0 mg/kg at zero hour.

TABLE 3

MEAN ± S.E. CHANGE IN T_b IN °C OF RATS TREATED EARLIER WITH ETHANOL (MEAN FALL = −2.1 ± 0.2°C IN 1.3 HR) FOLLOWING 1.0 OR 2.0 mg/kg RO 15-1788 GIVEN INTRAPERITONEALLY AT NADIR IN T_b

Treatment	15 Min	1.0 Hr	Δ Maximum	Latency (hr)
RO 15-1788 1.0 mg (N = 6)	−0.3 ± 0.1	−0.5 ± 0.2	−0.9 ± 0.2	(1.4 ± 0.1)
RO 15-1788 2.0 mg (N = 5)	−0.5 ± 0.1	−1.3 ± 0.2	−1.8 ± 0.3	(1.8 ± 0.2)

Maximum change in °C and latency is in time indicated.
Latency is hours to maximum decline.

TABLE 4

PERCENT OF RATS TREATED EARLIER WITH ETHANOL (MEAN FALL IN T_b = −2.1 ± 0.2°C IN 1.3 HR), DISPLAYING BEHAVIORAL SLEEP, AREFLEXIA AND INCOORDINATION AT 15 MIN AND 1.0 HR (TIME COURSE) AFTER 1.0 mg/kg (N=6) OR 2.0 mg/kg (N=5) RO 15-1788 WAS GIVEN INTRAPERITONEALLY AT THE NADIR IN T_b

	Sleep and Areflexia Time Course			Arousal and Reflex Intact	
Treatment	0 Min	15 Min	1 Hr	Onset	Complete
RO 15-1788 1.0 mg	100%	100%	100%	60% 5.0 hr	100% 6.0 hr
RO 15-1788 2.0 mg	100%	100%	100%	60% 5.0 hr	100% 6.5 hr
	Incoordination Time Course			Coordination	
	0 Min	15 Min	1 Hr	Onset*	Recovered
RO 15-1788 1.0 mg	100%	100%	100%	0% 6.0 hr	0% 7.0 hr
RO 15-1788 2.0 mg	100%	100%	100%	0% 6.0 hr	0% 7.0 hr

*Walking on rotorod but not to 3 min criterion.

RESULTS

As described previously (23,28) a dose of 4.0 g/kg of ethanol administered intraperitoneally to the rats maintained at the laboratory's temperature of 23–25°C produced a decline in mean colonic temperature (T_b) of 2.1 ± 0.2°C. As shown in Table 1, the maximal fall occurred at 1.3 ± 0.2 hr following the injection.

RO 15-1788 Treatment

As shown in Fig. 1, RO 15-1788 alone in a dose of 1.0 mg/kg did not exert a hypothermic effect; however, ethanol induced a hypothermia which did not differ from that observed when administered in combination with 1.0 mg/kg RO 15-1788 at intervals of 15 min and 1 hour after their injection. The maximal decline in temperature at 2.3 ± 0.2 hr (Table 1) following simultaneous treatment with the combined drugs was significantly different, $F(2,10)=8.75$, $p<0.01$, than that after ethanol was given alone.

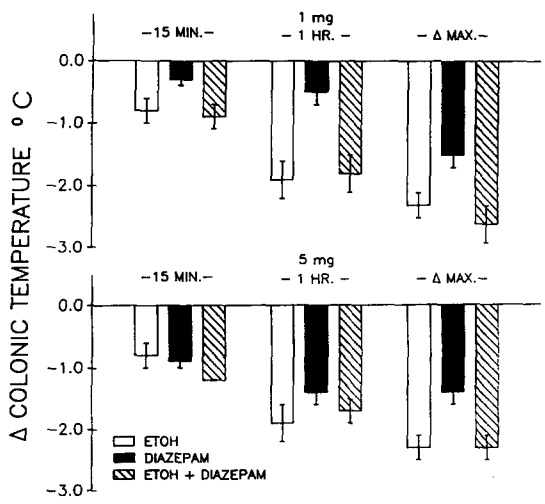

FIG. 3. Mean ± S.E. changes in °C of colonic temperature at 15 min, 1.0 hr as well as maximum shift in T_b under three conditions: ethanol (ETOH) administered intraperitoneally alone in a dose of 4.0 g/kg; diazepam injected intraperitoneally alone in a dose of 1.0 mg/kg (top); and 5.0 mg/kg (bottom); and same doses of ETOH plus diazepam administered simultaneously.

In relation to behavioral sleep, areflexia and motor incoordination produced by ethanol, RO 15-1788 failed to antagonize these responses. As presented in Table 2, RO 15-1788 tended to delay the recovery of ethanol-induced motor impairment. At the 6-hour interval after injection of ethanol alone, motor incoordination persisted in 40% of rats. However, when all ethanol-treated rats had recovered their coordination at 7 hours, those given RO 15-1788 plus ethanol were unable to remain on the rotorod to the criterion of 3 minutes (Table 2).

The time course of effects of the two doses of RO 15-1788 at the maximal point of ethanol's effect is portrayed in Fig. 2. The hypothermic response induced by ethanol was not reversed by the benzodiazepine receptor antagonist but rather enhanced by the drug. The higher dose evoked a temperature fall approximately double that of the lower; in some rats, the core temperature declined nearly 4.0°C (Fig. 2) reaching less than 33°C. At the 15 min and 1.0 hour interval and at the point of maximal fall, temperature following RO 15-1788 was significantly lower than that after ethanol alone. As shown in Table 3, this enhancement was dose-dependant for the 1.0 mg/kg dose, $F(3,28)=9.55$, $p<0.01$, as well as the 2.0 mg/kg dose, $F(3,25)=31.55$, $p<0.01$.

Table 4 presents the effects of both doses of RO 15-1788, injected at the nadir in T_b, in terms of the behavioral and motor changes induced by ethanol. The rats' arousal from sleep and recovery of their righting reflex were complete at the 6.0 hr interval in all animals. However, at the end of the recording period, 7.0 hr after the injection of ethanol, the rats given either dose of RO 15-1788 were unable to negotiate the rotorod to a criterion of 3.0 min. This is in contrast to the recovery of motor coordination in all rats given ethanol alone (Table 2).

TABLE 5

MEAN ± S.E. MAGNITUDE OF CHANGE IN BASELINE CORE TEMPERATURE (T_b) IN °C OF RATS AT 15 MIN AND 1.0 HR AFTER INTRAPERITONEAL ETHANOL OR INTRAPERITONEAL DIAZEPAM (DOSE IN mg/kg) OR BOTH

Treatment	15 Min	1.0 Hr	Maximum Change	Hr to Nadir
ETOH (N=5)	−0.8 ± 0.2	−1.9 ± 0.1	−2.3 ± 0.2	1.7 ± 0.0
Diazepam 1.0 mg (N=6)	−0.3 ± 0.0	−0.5 ± 0.2	−1.5 ± 0.2	4.3 ± 0.8
Diazepam 5.0 mg (N=6)	−0.9 ± 0.1	−1.4 ± 0.2	−1.4 ± 0.2	0.8 ± 0.0
ETOH + Diazepam 1.0 mg (N=6)	−0.9 ± 0.2	−1.8 ± 0.3	−2.6 ± 0.3	3.0 ± 0.6
ETOH + Diazepam 5.0 mg (N=6)	−1.2 ± 0.0	−1.7 ± 0.3	−2.3 ± 0.2	2.3 ± 0.5

Maximum decline in T_b and hours to nadir are shown.

Diazepam Treatment

Although diazepam exerted a dose-dependent hypothermic effect on its own, at 15 min, $F(1,11)=216$, $p<0.01$, and at 1.0 hour, $F(1,11)=60.75$, $p<0.01$, the benzodiazepine receptor antagonist did not synergize nor potentiate the hypothermia induced by ethanol given simultaneously (Table 5). This in contrast to the effects of RO 15-1788. As shown in Fig. 3 no differences in T_b were observed at the 15 min and 1.0 hour intervals or at the time of maximum change, when ethanol was administered alone or in combination with diazepam given in the dose of 1.0 or 5.0 mg/kg. However, the maximum decline in T_b after the 1.0 or 5.0 mg/kg doses of diazepam was less intense than that after ethanol was given alone, $F(1,10)=43.64$, $p<0.001$; $F(1,10)=55.23$, $p<0.001$.

Although the higher dose of diazepam alone evoked sleep, areflexia and motor incoordination in the rats, recovery occurred in less than half the time than that after ethanol alone. As shown in Table 6, diazepam in combination with ethanol prolonged the recovery of all the responses: arousal from sleep and righting reflex required in 67% of the rats between 4–5 hr in contrast to 100% at 4 hr after ethanol alone. Only 50% of the rats recovered motor coordination after 1.0 mg/kg diazepam at 6.0 hr and at 7.0 hr, after the 5.0 mg/kg dose in contrast to 100% recovery at 6.0 hr after ethanol alone (Table 6).

When diazepam in either dose was injected at the nadir in temperature, the hypothermia induced by ethanol was not significantly potentiated at the 15 min and 1.0 hour intervals (Table 7). As shown in Fig. 4, diazepam in both doses prolonged the poikilothermic effect of ethanol which was significant at the 4.0, $F(2,14)=36.47$, $p<0.01$, and 5.0 hour intervals, $F(2,14)=26.06$, $p<0.01$. During this time course, however, there was no difference between the lower and higher doses of diazepam. In contrast, a dose-dependent delay in the recovery of motor coordination was produced by both doses of the drug (Table 8). Following the lower dose of diazepam, 60% of the rats recovered their motor coordination 7.0 hr after the injection of ethanol, but only 20% of rats recovered after the higher dose of the drug was administered.

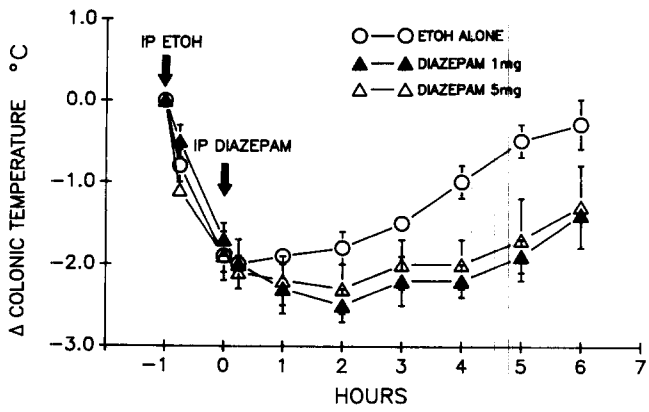

FIG. 4. Mean ± S.E. deviation in °C of colonic temperature over time in hr of rats given 4.0 g/kg ETOH intraperitoneally (ETOH ALONE) at −1 hour, or followed by diazepam injected intraperitoneally in doses of 1.0 or 5.0 mg/kg at zero hour.

DISCUSSION

In the context of the specific autonomic and behavioral impairments induced by ethanol, the present experiments show that diazepam and RO 15-1788 do not act as an agonist and antagonist, respectively. Although differential effects of diazepam and RO 15-1788 on the characteristic poikilothermia and other responses caused by ethanol are evident (23,28), both compounds in the doses used evoke effects generally in the same direction. Although our findings and those of others (9) demonstrate little or no effect on temperature of RO 15-1788 alone, a low dose of the BZ antagonist given intraperitoneally to the mouse reportedly may induce hypothermia (32). Further, RO 15-1788 given in a low dose exerts other effects on autonomic functions including bradycardia and a hypotensive response (31).

Previously it was shown that although RO 15-1788 does not alter body temperature, in a high dose it can block the typical fall in body temperature induced at room temperature by a benzodiazepine or a β-carboline, an inverse agonist of BZ receptors (37). RO 15-1788 also antagonizes the sedative effect of diazepam and other BZ agonists but not that produced either by a barbiturate (5) or ethanol (5,41). In fact, neither the intoxication-like state

TABLE 6

PERCENT OF RATS DISPLAYING BEHAVIORAL SLEEP, AREFLEXIA AND INCOORDINATION AT 15 MIN AND 1.0 HR (TIME COURSE) AFTER INTRAPERITONEAL ETOH INTRAPERITONEAL DIAZEPAM (DOSE IN mg/kg) OR BOTH

	Sleep and Areflexia Time Course		Arousal and Reflex Intact	
Treatment	15 Min	1.0 Hr	Onset	Complete
ETOH (N=5)	100%	100%	100% 2.5 hr	100% 4.0 hr
Diazepam 1.0 mg (N=6)	0%	0%	—	—
Diazepam 5.0 mg (N=6)	67%	67%	100% 1.4 hr	100% 2.0 hr
Diazepam 1.0 mg + ETOH (N=6)	100%	100%	50% 3.0 hr	67% 4.0 hr
Diazepam 5.0 mg + ETOH (N=6)	100%	100%	83% 4.0 hr	67% 5.0 hr
	Incoordination Time Course		Coordination	
	15 Min	1.0 Hr	Onset*	Complete
ETOH (N=5)	100%	100%	50% 4.0 hr	100% 6.0 hr
Diazepam 1.0 mg (N=6)	0%	0%	—	—
Diazepam 5.0 mg (N=6)	100%	100%	50% 1.5 hr	100% 2.0 hr
Diazepam 1.0 mg + ETOH (N=6)	100%	100%	67% 6.0 hr	50% 6.0 hr
Diazepam 5.0 mg + ETOH (N=6)	100%	100%	50% 6.0 hr	50% 7.0 hr

*Walking on rotorod but not to 3.0 min criterion.

produced by different doses of ethanol, including the same dose (4.0 g/kg) used in the present study, nor the ethanol-withdrawal syndrome is reversed by several doses of RO 15-1788 (1, 6, 21, 33). Similarly, in the human subject, RO 15-1788 in doses of 0.1–0.2 mg/kg does not counteract the intoxication induced by ethanol (14), and when given to volunteers in a dose of 0.5 mg, the BZ antagonist fails to alter the sedative effects of ethanol (20). Although the dose of RO 15-1788 administered to the individuals is relatively low in these cases, their lack of efficacy against ethanol coincides with the findings in rodents. Interestingly, the reversal by the partial inverse BZ receptor agonist, RO 15-4513, of ethanol's CNS depressant effects and induction of intoxication-like behavior can be antagonized by RO 15-1788 (6,15).

Other studies show that RO 15-1788 serves to potentiate the effect of ethanol in terms of an animal's acceptance of punishing shock (2,3). Further, a dose of 25 mg/kg RO 15-1788 only partially antagonizes the hypothermia at ambient temperature and incoordination produced in the mouse by chlordiazepoxide. This same dose not only fails to block the hypothermia induced by ethanol but potentiates the incoordination following ethanol (9). In this connection, diazepam in doses of 2.5–10 mg/kg potentiates hypothermia in rats exposed prenatally to ethanol (36), suggesting that ethanol exposure in utero exerts long-term effects on the thermoregulatory mechanisms influenced by a BZ receptor system in the brain. Taken together, it is apparent from these observations that this BZ receptor antagonist interacts functionally with ethanol through a complex mechanism within CNS neurons (8,38).

In related investigations, RO 15-1788 given in a high dose can exhibit intrinsic behavioral activity on its own (13) and may exert an effect paralleling that of a BZ receptor agonist (35). In terms of the mechanism of action of RO 15-1788, therefore, the drug may

TABLE 7

MEAN ± S.E. CHANGE IN T_b OF RATS TREATED EARLIER WITH ETHANOL (MEAN FALL = −1.8 ± 0.1°C IN 1.0 HR) FOLLOWING 1.0 OR 5.0 mg/kg DIAZEPAM GIVEN INTRAPERITONEALLY AT NADIR IN T_b

Treatment	15 Min	1.0 Hr	Δ Maximum	Latency (hr)
Diazepam 1.0 mg (N=5)	−0.4 ± 0.3	−0.6 ± 0.3	−1.1 ± 0.2	(1.7 ± 0.5)
Diazepam 5.0 mg (N=5)	−0.2 ± 0.2	−0.3 ± 0.3	−0.7 ± 0.2	(1.2 ± 0.4)

Latency is hours to maximum decline.

TABLE 8

PERCENT OF RATS TREATED EARLIER WITH ETHANOL (MEAN FALL IN T_b = −1.8 ± 0.1°C IN 1.0 HR), DISPLAYING BEHAVIORAL SLEEP, AREFLEXIA AND INCOORDINATION AT 15 MIN AND 1.0 HR (TIME COURSE) AFTER 1.0 OR 5.0 mg/kg DIAZEPAM WAS GIVEN INTRAPERITONEALLY AT THE NADIR IN T_b

	Sleep and Areflexia Time Course			Arousal and Reflex Intact	
Treatment	0 Min	15 Min	1.0 Hr	Onset	Complete
Diazepam 1.0 mg	100%	100%	100%	100% 3.0 hr	100% 5.0 hr
Diazepam 5.0 mg	100%	100%	100%	100% 4.0 hr	100% 5.0 hr
	Incoordination Time Course			Coordination	
	0 Min	15 Min	1.0 Hr	Onset*	Complete
Diazepam 1.0 mg	100%	100%	100%	80% 5.0 hr	60% 6.0hr
Diazepam 5.0 mg	100%	100%	100%	100% 5.0 hr	20% 6.0 hr

*Walking on rotorod but not to 3.0 min criterion.

act as a mixed agonist at a high dose and antagonist or inverse agonist at a low dose. However, either action of the compound depends on both the condition of the animal during its administration and the specific function being tested (6,39). For example, RO 15-1788 in a low dose may emulate a partial inverse agonist of the BZ receptor and block the anxiolytic effect of ethanol in a behavioral test (4). Alternatively, RO 15-1788 in doses of 1.0–2.0 mg/kg evokes a pattern of behavior in mice opposite to that produced by a BZ agonist (30), thus corroborating its role as an inverse agonist as well as antagonist of BZ receptors (13). Although RO 15-1788 exerts no effect alone on the firing rate of neurons in the substantia nigra (40), ethanol nonetheless seems to enhance GABA-mediated neurotransmission. Although RO 15-1788 reportedly enhances BZ binding to the GABA-B_2 ionophore receptor complex (7), it does not influence the binding characteristics of RO 15-1788 to the BZ receptor (16).

In summary, diazepam and ethanol possibly share the same functional substrate and anatomical site of action in the brain to produce hypothermia. Alternatively, RO 15-1788 apparently acts at a different locus perhaps at the level of signal transduction in brainstem neurons to potentiate ethanol-induced hypothermia. On the other hand, both drugs serve to augment the magnitude of incoordination induced by ethanol. This implies a common central mechanism underlying the motor impairment induced by ethanol which clearly is distinguishable from autonomic incapacitation. What is particularly interesting is the fact that diazepam and RO 15-1788 do not in themselves necessarily act as agonist and antagonist, respectively. Rather their actions, at least within the purview of the present experiments, are not pharmacologically opposite in terms of the distinct autonomic and motor effects produced by ethanol.

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