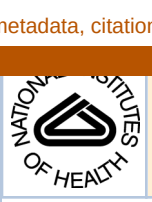

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Comparison of the CNS Effects Induced by TRH and Bicuculline After Microinjection into Medial Septum, Substantia Nigra and Inferior Colliculus: Absence of Support for a GABA Antagonist Action for TRH[1]

GEORGE R. BREESE, **GERALD D. FRYE**[2], **THOMAS J. McCOWN**, and **ROBERT A. MUELLER**

Departments of Psychiatry, Pharmacology, and Anesthesiology, The Biological Sciences Research Center and the Center for Alcohol Studies, University of North Carolina School of Medicine Chapel Hill, NC 27514

Abstract

Antagonism of ethanol-induced depression of locomotion was observed after intracisternal injection of thyrotropin releasing hormone (TRH) and bicuculline methiodide (BICM), as well as after microinjection of these drugs into the medial septum. The present investigation compared the behavioral and physiological consequence of administering TRH and BICM into the medial septum, inferior colliculus and substantia nigra to quantitate the similarities between these compounds. BICM produced a major increase in locomotor activity when injected into the medial septum and stereotypies when injected into the substantia nigra, suggesting that GABA-containing neurons have widespread influences on motor function. The wild running and seizure activity observed after BICM injection into the inferior colliculus was also consistent with this latter view. The marked increase in rectal temperature observed when BICM was injected into the medial septum may also implicate GABAergic mechanisms in temperature control at this brain site. TRH produced no such behavioral or physiological changes when administered into these three sites. Thus, this work strongly suggests that TRH does not exert a widespread action as a GABA antagonist because TRH did not produce the same changes induced by BICM. The actions of BICM and TRH to antagonize ethanol-induced depression when microinjected into the medial septum suggests that this brain area may be a critical site for the depressant action of ethanol.

Keywords

Site injection; Inferior colliculus; Medial septum; TRH; Substantia nigra; Bicuculline

Both thyrotropin releasing hormone (TRH) and the gamma-aminobutyric acid (GABA) antagonist, bicuculline, reduce the central nervous system depressant actions of ethanol [6,9]. Based upon the finding that GABA-mimetic compounds antagonized the action of TRH on ethanol, Cott and Engel [7] suggested that the analeptic effects of TRH were mediated via an inhibition of GABAergic systems. However, a GABA antagonist action of TRH remains to be established. Inhibition of glutamic acid decarboxylase (GAD) to reduce

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[2]Present address: Department of Medical Pharmacology and Toxicology, Texas A & M College of Medicine, College Station, TX 77843.

GABA synthesis as well as GABA receptor blockers characteristically induce CNS seizures [8,22]. Since TRH acts as an analeptic at doses which produce no seizure activity [6], the hypothesis that it might act as a GABA antagonist would only be viable if it were inhibiting GABA function at selected sites in brain.

While administration of TRH and bicuculline into the medial septum antagonized pentobarbital sleep [5,13,15], their effects on ethanol-induced narcosis after injection into this site is unknown. Previous work has demonstrated that blockade of GABA receptors in the substantia nigra results in motor disturbances and stereotypies [1]. In addition, bicuculline administration into the inferior colliculus causes wild running and seizures [10,11]. Since the physiological consequences of TRH alone are unknown at these sites, the pharmacological consequences of administering TRH and bibuculline into the substantia nigra, medial septum, and inferior colliculus were compared in an effort to determine if the behavioral of effects of TRH resembled those of a GABA antagonist, providing a means to test the hypothesis of Cott and Engel [7].

METHOD

General

Adult male Sprague-Dawley rats were used for all experiments (Charles River Laboratories, Somerville, MA). Rats were individually housed after surgical procedures under environmentally controlled conditions (0700 hr light, 1900 hr dark cycle, temperature 23–25°C; with continuous access to Wayne Lab Blox Laboratory chow and water) until used. Rats that received drugs intracisternally (IC, 25 μl) were housed four per cage prior to treatment.

All drugs administered intracisternally or microinjected into specific brain sites were dissolved in sterile 0.9% saline. Ethanol was administered IP at a concentration of 0.1 g/ml to minimize tissue irritation [24]. Thyrotropin releasing hormone (TRH) was donated by Abbott Laboratories, North Chicago, IL). The bicuculline methiodide was purchased from Pierce Chemical Co. (Rockford, IL). The absolute ethanol was purchased from AAPER Alcohol and Chemical Co. (Louisville, KY).

Data were evaluated with analysis of variance. Where significant treatment effects were observed ($p<0.05$), a Newman-Keuls test was used for post-hoc comparison of means.

Administration of Drugs into Brain

For intracisternal injection, TRH and bicuculline were introduced into the cistern magna in rats restrained by hand (see [9]). In order to allow microinjection of drugs into specific sites, cannulae were stereotaxically placed into the brain of anesthetized rats (sodium pentobarbital, 45 mg/kg) and secured to the skull with stainless steel screws and acrylic cement. Coordinates for the medial septum based upon the atlas of Paxinos and Watson [16] were for medial septum IA, 9.7, ML, 0.0, DV, 3.2; for substantia nigra IA, 3.7, ML, 2, DV, 2.0; for inferior colliculus IA, 0.2, ML, 1.5, DV, 6.0. A single cannula was placed into the medial septum whereas bilateral cannulae were implanted into the substantia nigra or inferior colliculus. Animals were allowed to recover for at least ten days before microinjection of drug or saline. The drugs were administered in a volume of 0.5 μl over a 5 min period through 33 gauge injection cannulae extending 1 mm below the guide tube [11]. During this period, the animals were hand held to allow minimal restraint. A 10 μl syringe (Precision Instrument Co., Baton Rouge, LA) driven by a Sage infusion pump (White Plains, NY) delivered the solution to the cannulae through polyethylene tubing. The injection cannulae remained in placed for one min after the end of the infusion. Rats received no more than two injections at least one week apart at a single site. Following experimental

procedures, the brains from rats were placed on a cryostat chuck and frozen on dry ice. Each brain was sectioned to allow localization of the cannula tip.

Locomotor Activity Measure

Locomotor activity was quantitated using doughnut-shaped activity monitors housed in sound-attenuated, fan-ventilated chambers lighted with a 7-watt lamp [12]. Interruption of one of the six photocells evenly spaced about a circular runway was recorded as an activity count. Counts were accumulated every 10 min over a 2 or 2.5 hour period. Rats were treated with saline or ethanol 5 min prior to injection of bicuculline methiodide, TRH or saline. Recording was initiated immediately after intracisternal injection or the removal of the injector. Activity was quantified between 1000 and 1500 hr.

Measurement of Rectal Temperature

Rectal temperature was recorded with a thermistor from Yellow Springs Instrument Co. (Yellow Springs, OH) inserted 4 cm into the rectum and held in place until a stable reading was obtained. Following infusion of drug or saline into the brain, rectal temperature was determined every 15 min for 1 hr and every 30 min for an additional 120 min. Room temperature was 24.4±0.1°C over the several experiments performed.

Observations

The effects of TRH and bicuculline on ethanol-induced locomotion, activity level, and rectal temperature were determined after administration into the medial septum. Infusion of bicuculline into the inferior colliculus has previously been demonstrated to induce seizure activity [11]. The sensitivity of the interior colliculus to convulsions was determined by infusing either bicuculline methiodide (10 ng/0.5μl) or TRH (1 μg/0.5 μl) bilaterally into this brain area at the rate of 0.1 μl/min for 5 min. The incidence of seizures and other physiological consequences were noted. Both drugs were also administered into the substantia nigra (bicuculline=0.6 μg; TRH=1 μg). The behavioral response (e.g., stereotypies, motor effects) were observed and recorded after injection into this latter site (see [1,11]). Rectal temperature was also measured after drug administration into the inferior colliculus and substantia nigra.

RESULTS

Effect of Intracisternally Administered TRH and Bicuculline Methiodide (BICM) on Ethanol-Induced Depression of Locomotion

Ethanol (2.5 g/kg, IP) produced a significant reduction in the activity of rats when compared to saline (Table 1). Intracisternally administered TRH at doses from 1.0 to 30 μg significantly reduced the effects of ethanol on locomotion and itself increased locomotion ([23], Fig. 1 legend). As previously reported [4], TRH (30 μg) in combination with ethanol produced activity greater than that for TRH alone (see Table 1 legend).

Bicuculline methiodide (BICM; 1 to 4 μg) administered intracisternally also antagonized the depression of locomotion induced by ethanol (Table 1). In the absence of ethanol, 2 of 4 rats had seizures at the 2 μg dose of BICM and all rats had seizures at the 4 μg dose (see Table 1 legend). Thus, it is difficult to ascertain whether the overall hyperactivity and seizures induced by BICM are responsible for the antagonism of ethanol-induced depression or whether specific sites unrelated to seizures might be responsible for the antagonism. Because of the seizures produced by BICM alone, it could not be determined whether activity was greater for BICM plus ethanol than for BICM alone.

Effects of TRH and Bicuculline Methiodide Administration into the Medial Septum on Ethanol-Induced Depression of Activity

When TRH and BICM were administered into the medial septum both compounds significantly antagonized the depression of locomotor activity induced by ethanol (Fig. 1). However, the manner in which the drugs affected sedation produced by ethanol differed (Fig. 1). The effect of TRH against ethanol-induced depression was apparent throughout the 120 min period of recording. However, the action of BICM against ethanol was greater in magnitude and of shorter duration (approximately 40 min) than that for TRH. Following the increase in activity, the ethanol-BICM-treated animals quickly reverted to the level of sedation observed with ethanol alone (Fig. 1).

Effects of TRH and Bicuculline Methiodide Administered into the Medial Septum on Activity and Body Temperature

In the absence of ethanol, a major difference in the physiological effects of TRH and bicuculline were observed when these compounds were administered into the medial septum. Even though all doses of TRH administered into the medial septum are capable of antagonizing ethanol-induced depression of locomotion (unpublished data), none of these doses of TRH alone induced a significant effect on locomotor activity (Table 2). Furthermore, tremor, body shaking and the piloerection observed after intracisternal injection of TRH [3,6] were not apparent after intraseptal TRH treatment. In contrast to the absence of an effect of TRH, all doses of BICM (0.45–1.8μg) administered into the medial septum caused a significant increase in locomotor activity (Table 2). This activity was characterized by increased coordinated movement and sniffing. No seizure-like activity was observed at these doses of BICM administered into the medial septum.

In addition to the change in locomotor activity, BICM produced a marked increase in rectal temperature (Table 2). The duration of this physiological effect was approximately 90 min for the 1.8 μg dose (data not shown). The maximal change and duration of this response was dose-related (Table 2). TRH produced no change in temperature when administered into the medial septum (Table 2).

Comparison of TRH and Bicuculline Methiodide Administered into the Inferior Colliculus on Seizure Activity

In accord with previous results [11], BICM (10 ng) infused into the inferior colliculus induced wild running and clonictonic seizure activity (Table 3). The relative sensitivity of this brain area was considerably greater than that of the medial septum where no seizure-like response was observed after BICM treatment (1.8 μg). BICM administration into the inferior colliculus did not significantly increase rectal temperature even though mild seizures and increased locomotor activity persisted for at least 45 to 60 (Table 3). Administration of TRH (1 μg) into the inferior colliculus produced no seizures or motor disturbances and did not significantly alter rectal temperature (Table 3, legend). Thus, the difference in action of these two compounds is further delineated at this brain site.

Comparison of TRH and Bicuculline Methiodide Effects on Motor Function When Administered into the Substantia Nigra

Previous studies have demonstrated that BICM administration into the substantia nigra can induce characteristic changes in motor function [1] and if sufficient dose is injected can induce seizure activity [8,11]. The effects of TRH and BICM were compared in the substantia nigra at doses having effects against ethanol-induced depression of locomotion when administered into the septum. In accord with previous observations, bicuculline induced distinctive stereotyped behaviors after nigral administration (see Table 4 for

detailed description). In contrast, TRH produced no such changes in behavior or motor function, even though TRH increases motor activity when given intracisternally (Table 1; [23]). Neither drug altered rectal temperature at 30 min after microinjection into the substantia nigra (Table 4).

DISCUSSION

In confirmation of earlier results, both TRH and BICM administered intracisternally were found to antagonize ethanol-induced depression (see [6,9]. Whereas BICM induced seizures at the 2 and 4 μg doses when given in the absence of ethanol, none of the TRH doses produced this response. In spite of this general difference in the action of these compounds after intracisternal injection, infusion of both compounds into the medial septum antagonized ethanol depression of locomotion, without eliciting seizure activity. Previous studies have indicated that TRH can act in the medial septum to antagonize pentobarbital sleep [13,15] as can bicuculline [5]. These observations provided evidence that the medial septum may be an important site of action for drugs which antagonize ethanol and other CNS depressants.

The common pharmacological action of TRH and BICM against ethanol in the medial septum, at first, appeared consistent with the suggestion of Cott and Engel [7] that TRH could be acting by inhibiting GABA function. In order to pursue this view, it was reasoned that if TRH were acting to inhibit GABA function at one site, then when administered into sites where a GABA antagonist caused physiological changes, TRH should have an effect similar to that of bicuculline. However, such studies in the medial septum, inferior colliculus and substantia nigra did not support the conclusion that TRH was having a GABA antagonist action.

The first site where distinctly different physiological consequences of TRH and BICM administration were found was the medial septum. TRH produced no discernible locomotion, tremor or piloerection when administered into this site. In contrast, BICM administered into the septum produced a major increase in locomotion and a marked rise in rectal temperature. It is difficult to determine with certainty whether the increase in locomotion induced the hyperthermia, although our impression is that the magnitude of the temperature change could not be totally associated with this increased activity. This view is based upon the observation that injection of bicuculline into the inferior colliculus resulted in continuous motor activity (seizures), but induced no significant change in rectal temperature. Since previous studies have implicated the medial septum in temperature control mechanisms [19,20], it would be of considerable importance to determine if GABAergic fibers at this site are critical for the maintenance of this homeostatic function.

Two other brain sites where microinjection of GABA antagonists have been shown to alter CNS function also were utilized to compare the effects of TRH with bicuculline. Administration of BICM into substantia nigra produced stereotypies, running, and gnawing [1]. While this response would seem to implicate GABAergic neurons in these responses, the significance of a GABA mechanism is confused by the observation that muscimol produces a similar response when administered at this site [18]. However, bicuculline does produce turning opposite to that of muscimol when injected unilaterally [17], indicating different mechanisms of action for these drugs in the substantia nigra. Regardless, TRH was without effect when administered into the substantia nigra.

The inferior colliculus is usually associated with integration of auditory impulses. Recently, McCown *et al.* [14] have demonstrated that stimulation of the inferior colliculus with low level electrical current can induce wild running. In agreement with our earlier results [11],

infusion of bicuculline into the inferior colliculus induced wild running and then convulsions. Frye *et al.* [10,11] have demonstrated that muscimol into this area of brain can block the seizures induced by sound in ethanol-dependent rats. Together, these data suggest that a GABAergic system is associated with this area of brain and that it can influence motor function. However, it remains to be determined how GABAergic mechanisms might play a role in auditory function. The finding by Tawil *et al.* [21] that spinal cord neurons influence neural activity in the inferior colliculus may be relevant to the present results.

In summary, these data suggest that the medial septum plays an important role in the analeptic action of TRH and bicuculline, because microinjection of these drugs into this site antagonized the ethanol-induced depression of locomotion. However, the different physiological actions observed when TRH and bicuculline were administered into the medial septum, substantia nigra and inferior colliculus suggest that TRH does not antagonize GABA function to produce its analeptic action. This conclusion would be consistent with the work of Bjorkman *et al.* [2], who found no relationship between GABA function and the action of TRH to increase temperature and cause tremors. The physiological consequences observed when bicuculline methiodide was administered into these sites suggest that GABA-containing neurons can influence motor function, temperature mechanisms and may be involved in central circuitry associated with auditory output.

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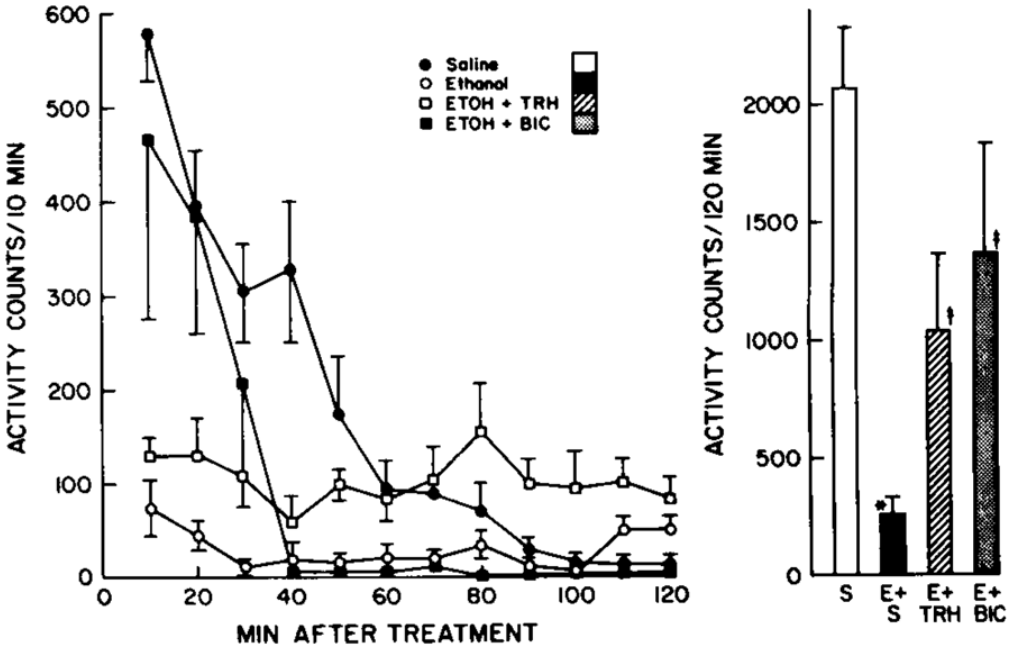

FIG. 1.

Effect of bicuculline and TRH administration into the medial septum on locomotor activity: Overtime (left side) and on total accumulated counts (right side). Total counts for TRH (1μg) and bicuculline (1.8μg) are presented in Table 2. S = saline, E = ethanol and BIC = bicuculline methiodide. The dose of ethanol was 2.5 g/kg given IP 5 min before the infusion of TRH, bicuculline methiodide, or saline. *p<0.001 when compared to saline treatment. †p<0.01 when compared to ethanol treatment.

TABLE 1

EFFECT OF INTRACISTERNALLY ADMINISTERED TRH AND BICUCULLINE ON ETHANOL-INDUCED DEPRESSION OF LOCOMOTOR ACTIVITY[†]

Treatment	Dose	Activity Response (counts/120 min)
Saline	—	1533 ± 147
Ethanol (E)	2.25 g/kg	437 ± 41
Ethanol + TRH	E + 3/μ.g	1585 ± 180[*]
Ethanol + TRH	E + 10 μg	3338 ± 480[*]
Ethanol + TRH	E + 30 μg	4845 ± 676[*]
Ethanol + Bicuculline	E +1$\mu.g$	1101 ± 77[*]
Ethanol + Bicuculline	E + 2μg	1266 ± 226[*]
Ethanol + Bicuculline	E + 4 μg	2050 ± 365[*]

[*] $p<0.01$ when compared to ethanol alone.

[†] TRH and Bicuculline doses were administered to saline and ethanol treated rats five minutes after receiving these injections. Bicuculline refers to bicuculline methiodide. Locomotor activity (counts/120 min) for TRH alone for each dose (n=at least 6) was: 3 μg=*2194 ± 356;* 10 μg=3082 ± 716; 30 μg=3223 ± 616. Locomotor activity (counts/120 min) for bicuculline alone for each dose (n=4) was: 1μg=1055 ± 135; 2 μg= 1260; 4 μg=not determined. All rats that received only the 4 μg dose of bicuculline intracisternally demonstrated seizure-like symptoms and died.

TABLE 2

EFFECTS OF TRH AND BICUCULLINE ADMINISTRATION INTO THE MEDIAL SEPTUM ON LOCOMOTOR ACTIVITY AND RECTAL TEMPERATURE[†]

Treatment	Dose (μg)	Locomotor Activity (counts/120 min)	Rectal Temperature (°C at 30 min)
Saline	—	1986 ± 333	37.5 ± 0.11
TRH	0.5	1734 ± 101	38.0 ± 0.23
TRH	1.0	2350 ± 471	37.7± 0.24
TRH	5.0	2450 ± 349	37.8± 0.29
Bicuculline	0.45	3785 ± 571[*]	38.9 ± 0.34[*]
Bicuculline	0.90	5713 ± 301[*]	39.0 ± 0.19[*]
Bicuculline	1.8	8557 ± 677[*]	40.1 ± 0.18[*]

[*] $p<0.05$ when compared to saline.

[†] Drugs were microinjected into brain as described in methods. Bicuculline refers to Bicuculline Methiodide (BICM). Locomotor activity counts were accumulated for 120 min. Rectal temperature was recorded 30 min after microinfusion in a separate group of rats; pre-infusion temperature was 37.7 ± 0.17°C. Number of rats per group was at least 5.

TABLE 3

EFFECT OF TRH AND BICUCULLINE ADMINISTRATION INTO THE INFERIOR COLLICULUS[†]

Treatment	Dose (μg)	Observed Response[*]
Saline	—	No apparent effect
TRH	1.0	No apparent effect
Bicuculline Methiodide	0.01	Wild running Clonic Seizures

[*] Temperature at 30 min after treatment (N = 5); Saline = 38.3 ± 0.1°C; TRH = 38.4 ± 0.08°C; BICM = 38.3 ± 0.3; (p>0.1 for all comparisons).

[†] Rats were infused with the indicated dose at a rate of 0.1 μl/min for 5 min. All rats receiving bicuculline had seizures. Each observation is at least 5 rats.

TABLE 4

EFFECTS OF TRH AND BICUCULLINE ADMINISTRATION INTO THE SUBSTANTIA NIGRA[†]

Treatment	Dose (μg)	Behaviors Observed[*]
Saline	—	No apparent effect
TRH	1.0	No apparent effect
Bicuculline Methiodide	0.6	Stereotyped behaviors Head bobbinig, sniffing, gnawing, wild running

[*] Temperature at 30 min after treatment: Saline = 38.0 ± 0.2; TRH = 38.4 ± 0.1; BICM = 38.2 ± 0.32; (p>0.1 when compared to Saline).

[†] Rats were observed for 30 min after microinjection into the substantia nigra (N = at least 5 rats).