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Inhibition of the Binding and the Behavioral Effects of Thyrotropin-Releasing Hormone (TRH) by the Triazolobenzodiazepines

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KOZLOWSKI, M. R. *Inhibition of the binding and the behavioral effects of thyrotropin-releasing hormone (TRH) by the triazolobenzodiazepines.* PHARMACOL BIOCHEM BEHAV **30**(1) 73–75, 1988.—The abilities of 4 triazolobenzodiazepines, adinazolam, alprazolam, estazolam and triazolam, to inhibit thyrotropin-releasing hormone (TRH) receptor binding and to antagonize the narcoleptic effects of TRH were examined. The IC50 values for inhibition of ^{3}H-3-methyl-His-2-TRH (MeTRH) binding ranged from 19 μM to 477 μM, and the Hill coefficient from 0.53 to 0.98. Similar ranges of values were obtained from benzodiazepines of other structural classes. Thus, the inhibition of TRH receptor binding by the triazolobenzodiazepines is similar to that produced by other types of benzodiazepines. Furthermore, the triazolobenzodiazepine, alprazolam, antagonized the narcoleptic effect of TRH. However, this action is not necessarily linked to its inhibition of TRH receptor binding since alprazolam also inhibited the narcoleptic effect of amphetamine.

Thyrotropin-releasing hormone (TRH) ^{3}H-MeTRH binding Triazolobenzodiazepines Pentobarbital-induced narcosis

THYROTROPIN-RELEASING hormone (TRH) acts directly upon the central nervous system to produce behavioral effects such as reduction of barbiturate-induced narcosis, and hypermotility [1,3]. These effects may be mediated by the TRH binding sites found in brain tissue [8,11].

Several benzodiazepine derivatives inhibit TRH binding to brain tissue [5, 7, 9, 10, 12]. However, the effect of the benzodiazepines that inhibit TRH binding on the behavioral and biochemical actions of TRH is complex. In some cases, the actions of TRH are inhibited by these benzodiazepines [4,5], but in other cases they are unaffected or even potentiated [7,13].

One class of benzodiazepine derivatives whose interactions with TRH have not been explored is the triazolobenzodiazepines (TBD's). The present study examines the effects of four TBD's on the receptor binding and behavioral effects of TRH.

METHOD

TRH Binding Assay

TRH receptor binding was measured using an adaptation of previously described techniques [8,11]. Frozen (−60°C) bovine hippocampus was thawed and homogenized for 30 sec in 50 volumes of 50 mM Tris-acetate buffer, pH 7.4, using a polytron homogenizer. The homogenate was centrifuged at 30,000×g for 30 min, the pellet resuspended in fresh buffer, and the centrifugation and resuspension steps repeated. Aliquots of the tissue were incubated with ^{3}H-MeTRH (57.8 Ci/mmol, New England Nuclear), and a solution of either a benzodiazepine unlabeled TRH or the vehicle (0.05% emulphor, 0.05% ethanol, 99.9% buffer). The incubation volume was 1 ml, the tissue concentration was 2 mg/ml, and the ligand concentration was 1 nM. All incubations were done in duplicate. The mixture was incubated for 6 hr at 3°C and the incubation terminated by filtration followed by 5 1-sec washes with ice-cold buffer. Radioactivity trapped by the filters was measured by scintillation counting. Non-specific binding was defined as that occurring in the presence of 1 μM TRH. These conditions yielded 80% specific binding and a K_i for TRH of 24 nM (data not shown), in agreement with the results of others [8,11].

Pentobarbital Narcosis

Groups of 5 mice, fasted for 18 hr, were dosed with TRH (0.32 mg/kg, IP), alprazolam (ALP; 0.32 mg/kg, PO), am-

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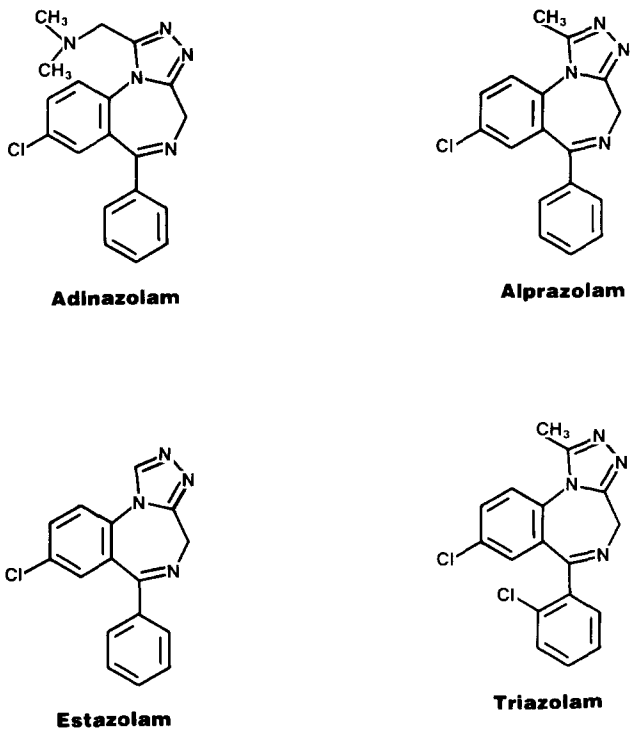

FIG. 1. Structures of the triazolobenzodiazepines tested.

phetamine (1.0 mg/kg, IP), or the vehicle for all of the drugs, saline (1 ml/kg, PO). Two other groups were given combinations of ALP (0.32 mg/kg, PO) and TRH (0.32 mg/kg, IP), or of ALP (0.32 mg/kg, PO) and amphetamine (1.0 mg/kg, IP). In the combination experiments, ALP was given simultaneously with amphetamine, but 55 min before TRH. The doses were chosen on the basis of preliminary studies (data not shown). ALP was given PO rather than IP so that the routes for the 2 drugs would be different when it was given with TRH or amphetamine. The rats were given an hypnotic dose of pentobarbital (55 mg/kg, IP) 5 min after TRH, or 1 hr after the other drugs. The interval from the loss of righting until its recovery was recorded.

RESULTS

TRH Receptor Binding

All 4 TBD's tested (Fig. 1), adinazolam (ADI), ALP, estazolam (EST), and triazolam (TRI), inhibited the binding of ^{3}H-MeTRH at micromolar concentrations. The range of IC50 values spanned more than an order of magnitude with the most potent compound being TRI and the least potent, EST (Table 1). The IC50 value for each drug had substantial amount of variability which was attributed to the difficulty of dissolving the drugs at these high concentrations and, thus, obtaining uniform concentrations between assays. Hill coefficients calculated from the inhibition data were close to 1 for ALP, EST and TRI, but less than 1 for ADI.

The effects of chlordiazepoxide (CHL), diazepam (DIA), flurazepam (FLU), and midazolam (MID), benzodiazepines of other structural classes, on ^{3}H-MeTRH binding were also examined for comparison with the TBD's. All compounds inhibited binding in the micromolar concentration range similarly to the TBD's. The order of potency was MID>CHL>FLU>DIA. Also similarly to the TBD's, the Hill coefficients varied within this group; for DIA and FLU the values were near 1, but CHL and MID they were less than 1.

TABLE 1

INHIBITION OF ^{3}H-MeTRH BINDING BY BENZODIAZEPINES

Compound	IC50 (μM)	Hill Coefficient
Triazolobenzodiazepines		
Adinazolam	223.3 ± 78.4	0.53 ± 0.06
Alprazolam	46.0 ± 28.8	0.98 ± 0.25
Estazolam	476.7 ± 178.9	0.79 ± 0.13
Triazolam	19.3 ± 0.5	0.74 ± 0.28
Other benzodiazepines		
Chlordiazepoxide	6.9 ± 1.8	0.62 ± 0.05
Diazepam	113.0 ± 58.4	1.01 ± 0.26
Flurazepam	67.3 ± 16.6	0.88 ± 0.07
Midazolam	3.3 ± 2.2	0.58 ± 0.22

All values are the mean ± standard error of 3 determinations.

TABLE 2

EFFECTS OF TRH, AMPHETAMINE, AND ALPRAZOLAM ON PENTOBARBITAL-INDUCED NARCOSIS

Treatment	Duration of Narcosis (min)
Saline	126 ± 12
TRH (0.32 mg/kg, IP)	85 ± 5*
Amphetamine (1.0 mg/kg, IP)	81 ± 13*
ALP (0.32 mg/kg, IP)	156 ± 12

*Significantly different from saline group; Dunnett t-test, $p<0.05$, $df=12$.

Results are the mean ± S.E.M. for 5 mice.

Pentobarbital Narcosis

TRH reduced the duration of pentobarbital-induced narcosis in mice (Table 2) as previously reported [3]. ALP alone did not alter the duration of narcosis to a statistically significant extent, although it produced a slight increase. ALP blocked the attenuation of narcosis by TRH (Table 3). In fact, in the animals given a combination of TRH and ALP, the duration of narcosis was longer than in the controls. This may reflect the slight increase in the duraction of narcosis produced by ALP alone. ALP had a similar reversing effect on the attenuation of pentobarbital-induced narcosis by amphetamine.

DISCUSSION

TRH receptor binding in brain is inhibited by the TBD's at micromolar concentrations, as it is for the other types of benzodiazepines tested (present results, [5, 7, 9, 10, 12]). Although it is impossible to draw any definitive conclusions about structure-activity relationships from the small number of TBD's tested, the substituent at position 1 (5a of the fused triazolo-ring) appears to have a large effect on potency.

TABLE 3

REVERSAL OF THE ANALEPTIC EFFECTS TRH AND AMPHETAMINE BY ALP

Treatment	Duration of Narcosis (min)
Saline	146 ± 7
TRH	106 ± 14
TRH + ALP	192 ± 9*
Amphetamine	107 ± 4
Amphetamine + ALP	170 ± 15†

*Significantly different from TRH alone; *t*-test with Bonferroni adjustment, $p<0.05$, $df=8$.

†Significantly different from amphetamine alone, *t*-test with Bonferroni adjustment, $p<0.05$, $df=8$.

Values and doses as in Table 2. For a more complete description of the treatments see the Method section.

Methyl (ALP, TRI) is better than dimethylaminomethyl (ADI), which is better than hydrogen (EST).

Although the Hill coefficients for ALP, EST, and TRI are close to 1, that for ADI is clearly less than unity. Low Hill coefficients have been reported for the inhibition of TRH binding by benzodiazepines [7,12]. In particular, Hill coefficients less than unity have been reported for CHL and MID as found in this study. However, another study did not find low Hill coefficients associated with the inhibition of TRH binding by several other benzodiazepines, as well as CHL [10]. The Hill coefficient found for CHL in the present report agrees with the former studies. However, our results go on to show that low Hill coefficients do not characterize the inhibition of TRH binding by benzodiazepines, but only the inhibition by specific benzodiazepines. Although, in this case too, the number of TBD's tested in this study is too small to determine which structural features underly the value of the Hill coefficient, it appears that the substituent at position 1 may again be important. Thus ADI, with a Hill coefficient less than 1, differs from ALP and EST, with Hill coefficients equal to 1, only in the substituent at this position (dimethylaminoethyl vs. methyl or hydrogen). Low Hill coefficients are suggestive of negative binding cooperativity or the presence of multiple binding sites [2]. The present results cannot distinguish between these, or other, alternatives.

The attenuation of barbiturate-induced narcosis produced by TRH (present results; [3]) is reversed by ALP at a dose which, by itself, produces only a slight, not statistically signficant, increase in narcosis. Taken together with the receptor binding results, these data suggest that ALP is a TRH antagonist, in agreement with some [4,5], but not all [7,13], literature reports examining other benzodiazepines and other functional endpoints. However, this interpretation of the data is confounded by the finding that ALP also reverses the analeptic effects of amphetamine. The apparent nonselectivity of the antagonism of analepsy by ALP casts doubt on its relationship to the inhibition of TRH receptor binding.

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