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Pharmacology of Pyrazolopyridines

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PATEL, J. B., J. B. MALICK, A. I. SALAMA AND M. E. GOLDBERG *Pharmacology of pyrazolopyridines*. PHARMACOL BIOCHEM BEHAV **23**(4) 675–680, 1985.—Pyrazolopyridines (PZP's) in general represent a chemically unique class of non-sedative anxiolytic agents. Tracazolate (ICI 136,753) is a member of pyrazolopyridine series that has shown anxiolytic properties in animal models. Tracazolate demonstrates a wider separation between sedative and therapeutic doses than do benzodiazepines. In addition, tracazolate appears to cause fewer adverse interactions than the benzodiazepines in combination with barbiturates and alcohol. In interaction studies, tracazolate potentiated both the antimetrazol and anticonflict effects of chlordiazepoxide. Pyrazolopyridines cause enhancement of both ^{3}H-flunitrazepam (^{3}H-FLU) and ^{3}H-GABA to their binding sites in brain. The enhancement of ^{3}H-FLU binding by PZP's and GABA are additive and reversed by bicuculline. The enhancement of ^{3}H-GABA binding by PZP's and benzodiazepines are additive and reversed by picrotoxin. It is hypothesized that the action of PZP's, and particularly tracazolate, may be related to their effects upon a GABA-stimulated chloride ionophore site. Finally, benzodiazepine antagonists (e.g., RO-15 1788) fail to reverse either the anxiolytic properties of ^{3}H-FLU enhancers or their ^{3}H-GABA binding enhancement effects. In contrast, benzodiazepine antagonists readily reverse the anxiolytic effects of benzodiazepines and non-benzodiazepines which cause ^{3}H-FLU displacement. These data suggest that tracazolate, a non-benzodiazepine, has a pharmacological profile suggestive of novel anxiolytic activity.

Pyrazolopyridines Tracazolate

SINCE the introduction of the first benzodiazepine, chlordiazepoxide, in 1960, many laboratories have been attempting to discover novel non-benzodiazepine anxiolytics that are devoid of the side effects of benzodiazepines i.e., sedation, alcohol potentiation and abuse liability. Recently, several distinct classes of non-benzodiazepine anxiolytics have been reported that meet at least some of these criteria. The comparative pharmacological profiles of these distinct classes have been described by Goldberg and associates [10]. Among these series are the pyrazolopyridines (e.g., tracazolate (ICI 136,753) [22]; cartazolate (SQ 65,396) [2]; etazolate (SQ 20009) [1]), triazolopyridines (e.g., CL 218,872) [15]; pyrazoloquinolines (e.g., CGS 9896) [31]; and piperazinyl pyrimidines (e.g., buspirone) [9]. Of these classes, triazolopyridines and pyrazoloquinolines displace labeled benzodiazepines from binding sites, whereas pyrazolopyridines,enhance benzodiazepine binding; piperazinyl pyrimidines do not significantly alter benzodiazepine binding *in vitro*. This report describes the pharmacological and neurochemical effects of tracazolate (ICI 136,753), a prototype compound of the pyrazolopyridine class (Fig. 1).

FIGURE 1

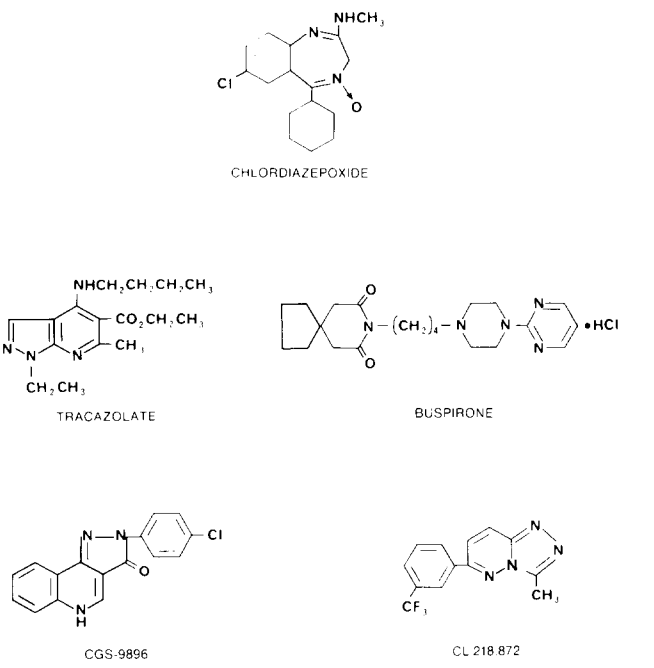

FIG. 1. Structures of tracazolate and representative anxiolytics from various chemical series.

GENERAL METHOD

The animals used in the following studies were male Wistar (Hilltop Laboratories, Scottdale, PA) rats (180–220 g) and male Swiss- Webster (HLA) mice (18–25 g). All tested drugs were suspended in an HPMC vehicle (0.1% Tween® 80, 0.5% hydroxypropyl methylcellulose in 0.9% NaCl). Drugs were administered in volumes of 5 ml/kg for rats and 20 ml/kg for mice.

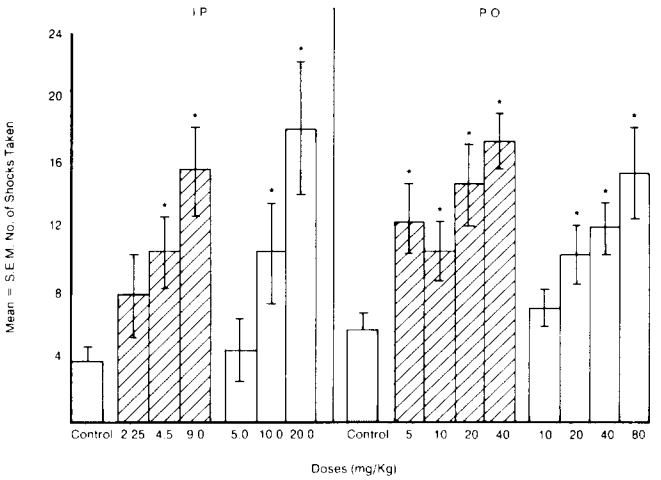

FIG. 2. Comparative effects of chlordiazepoxide (diagonally slanted line column) and tracazolate (open column) in the SSD test in rats. N=15–18/group. *$p<0.05$; Student's t-test.

NEUROPHARMACOLOGICAL STUDIES

Anxiolytic Activity

Anxiolytic effects were investigated in rats using the shock-induced suppression of drinking (SSD) test, which is a modification of a test originally reported by Vogel and coworkers [28]. The details of the test have been described previously [22]. The anticonflict activity of tracazolate and chlordiazepoxide, following both intraperitoneal and oral administration, are presented in Fig. 2. Both chlordiazepoxide and tracazolate produced a dose-related statistically significant ($p<0.05$) increase in the mean number of shocks received. In these studies tracazolate appeared to be one-half to one-quarter the potency of chlordiazepoxide (CDP). At equieffective oral doses both agents had a similar duration of activity (data not shown). Tolerance to the anxiolytic activity of either CDP or tracazolate did not develop following 12 days of drug administration.

It should be mentioned that tracazolate lacks significant anticonflict activity in the Geller-Seifter conflict model [8], whereas, chlordiazepoxide, as expected, significantly increased punished responding [22].

Tracazolate has also been shown to exhibit anxiolytic activity in the Mouse Exploratory Conflict procedure, which was a modification of a procedure originally described Boissier and Associates [4]. In this procedure, the anticonflict MED (minimal effective dose; i.e., the lowest dose producing significant disinhibition) for chlordiazepoxide and tracazolate was 15 and 25 mg/kg, PO, respectively [17].

Anticonvulsant Activity

The ability of an agent to prevent or antagonize pentylenetrazol, bicuculline or sound induced seizures was determined in mice. The details of the pentylenetetrazol and bicuculline procedures were described previously [22]. In the audiogenic seizure studies, DBA-2J mice (20–22 days of age) were given drug intraperitoneally 30 minutes prior to being subjected to the sound produced by an electric bell (116–118 dB for 60 sec).

Table 1 summarizes the results obtained in mice using these different experimental seizure procedures. As expected, chlordiazepoxide produced anticonvulsant effects in all of these types of seizures at the ED_{50} range of 1.0–3.0 mg/kg, IP. Tracazolate was considerably weaker (10 to 20 times) and exhibited its best effects against audiogenic seizures (ED_{50}=14.5 mg/kg). In contrast, cartazolate and etazolate are nearly devoid of anticonvulsant effects at nontoxic doses and, in fact, cartazolate induces convulsions at high doses.

TABLE 1

MEDIAN EFFECTIVE DOSES (ED_{50}-mg/kg. I.P.) for ANTICONVULSANT ACTIVITY IN MICE

Convulsants	Chlordiazepoxide	Tracazolate
Pentylenetetrazol (82.0 mg/kg S.C.)	3.0	27.5
Bicuculline (2.1 mg/kg S.C.)	0.9	28.0
Audiogenic Seizures (116 dB sound)	2.7	14.5

TABLE 2
SEDATIVE LIABILITY INDEXES FOR CHLORDIAZEPOXIDE AND TRACOZOLATE IN MICE AND RATS

	Rotorod impairment (ED_{50} mg/kg p.o.)[a]		Anticonflict activity (MED mg/kg p.o.)[b]		Sedative liability index rotorod ED_{50}/conflict MED	
Drug	Mouse	Rat	Mouse	Rat	Mouse	Rat
Chlordiazepoxide	18.9	37.4	15	5	1.3	7.5
Tracazolate	117.1	400	25	20	4.7	20

[a]ED_{50} calculated by the method of Litchfield and Wilcoxon 1949

[b]MED = minimal effective dose (i.e. the lowest dose producing a statistically significant increase in the number of shocks taken)

TABLE 3
INTERACTION OF CHLORDIAZEPOXIDE AND TRACAZOLATE WITH ETHANOL IN MICE

	Rotorod performance	
Treatment[a]	Dose (mg/kg. p.o.)	% Ataxia
Control (HPMC)	—	8.3
Chlordiazepoxide	2.5	33.3
	5.0	33.3
	10.0[b]	50.0
	20.0	100.0
Tracazolate	12.5	0.0
	25.0	0.0
	50.0	16.7
	100.0	16.7

[a]Treatments were administered 30 min. prior to ethanol (1.2 g/kg. i.p.).

[b]MED (i.e., the lowest dose of drug causing impairment in 50% or more of the mice tested).

Sedative Liability

To determine the comparative effects on sedation and motor incoordination, tracazolate and standard reference anxiolytics were evaluated in the forced motor activity or rotorod test [13]. In these studies, rats or mice were trained

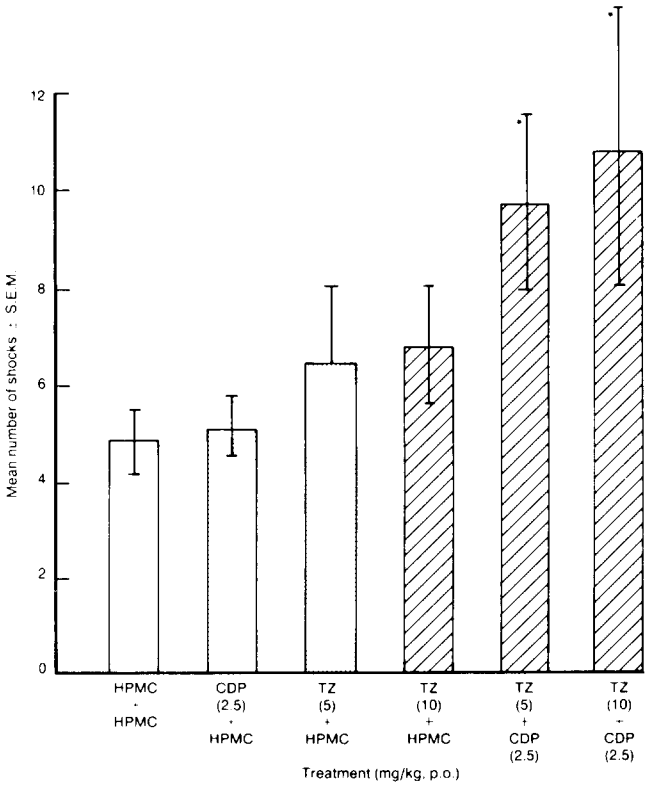

FIG. 3. Effects of tracazolate and chlordiazepoxide alone and in combination in the SSD test in rats. *$p<0.05$; Student's t-test.

to maintain themselves for at least 1 minute on a rotating rod (5 rpm for mice, 6 rpm for rats). Subjects were treated orally with test agents and were retested for their ability to maintain themselves on the rotorod at various times post-drug administration.

The comparative activity of tracazolate and chlordiazepoxide in the rotorod test is summarized in Table 2. Chlordiazepoxide produced ataxia at relatively low doses; its ED_{50}'s were 18.9 and 37.4 mg/kg PO in mice and rats, respectively (Table 2). Tracazolate, on the other hand, was markedly less sedating, exhibiting an ED_{50} for rotorod impairment of 117 mg/kg PO in mice and it failed to significantly impair rotorod performance in rats at any dose up to the highest dose tested, 400 mg/kg, PO. As shown in Table 2, tracazolate has a wider separation between doses that produce anticonflict and sedative effects than chlordiazepoxide and would be predicted to possess less sedation at anxiolytic doses than CDP in man.

Interaction with Ethanol

When evaluating potential non-benzodiazepine anxiolytics, it is essential to determine the comparative potentiation liability of benzodiazepines and agents like tracazolate when they are administered in combination with ethanol. Therefore, mice were treated with a test compound prior to administration of ethanol and then retested on the rotorod at various intervals. Table 3 summarizes the results of these tests with chlordiazepoxide and tracazolate. It can be seen from Table 3 that acute administration of chlordiazepoxide produced a dose-related enhancement of ethanol-induced rotorod impairment; its MED (i.e., the lowest dose of drug producing impairment in 50% or more of the mice tested) was 10 mg/kg, PO. In contrast, tracazolate failed to significantly potentiate ethanol-induced impairment of rotorod performance even at the highese dose tested (100 mg/kg, PO). These data indicate that tracazolate should be much less likely than the benzodiazepines to potentiate the effects of ethanol in man. Similarly, chlordiazepoxide produced a significant prolongation of barbital sleeptime at doses as low as 5 mg/kg, PO. In contrast, tracazolate failed to potentiate the sleeptime induced by barbital at doses as high as 80 mg/kg, PO [17].

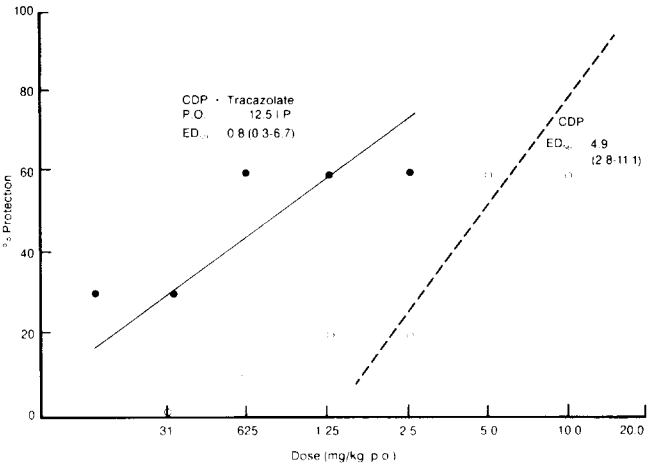

FIG. 4. Effects of chlordiazepoxide alone and in combination with tracazolate on metrazol-induced convulsions in mice.

Combination Studies with Benzodiazepines

In view of tracazolate's unique neurochemical profile, (enhancement of benzodiazepine to its binding sites in brain), studies were performed to determine whether tracazolate and chlordiazepoxide would interact pharmacologically.

The results of such a drug combination study are presented in Fig. 3. For the purpose of this experiment, ineffective doses of both tracazolate (5 and 10 mg/kg) and chlordiazepoxide (2.5 mg/kg) were administered 30 minutes prior to the test. Neither chlordiazepoxide (2.5 mg/kg, PO) nor tracazolate (5 and 10 mg/kg, PO) produced significant anxiolytic activity when given alone. However, significant anticonflict activity was seen with both doses of tracazolate when combined with chlordiazepoxide.

Likewise, the effects of drug combination were evaluated using the antagonism of metrazol-induced convulsion test in mice. In this study, chlordiazepoxide exhibited an ED_{50} for antagonism of metrazol convulsions of 4.9 mg/kg, PO. In the presence of an inactive dose of tracazolate, the dose response curve for antagonism of metrazol-induced convulsions was shifted significantly to the left. In combination with tracazolate the ED_{50} for chlordiazepoxide was 0.8 mg/kg, PO (Fig. 4). These findings indicate that tracazolate can potentiate both the anticonvulsant and anticonflict actions of chlordiazepoxide.

NEUROCHEMICAL STUDIES

Effects on [^{3}H] Flunitrazepam Binding

The potency of several benzodiazepines in displacing [^{3}H]-benzodiazepines from their binding sites correlates

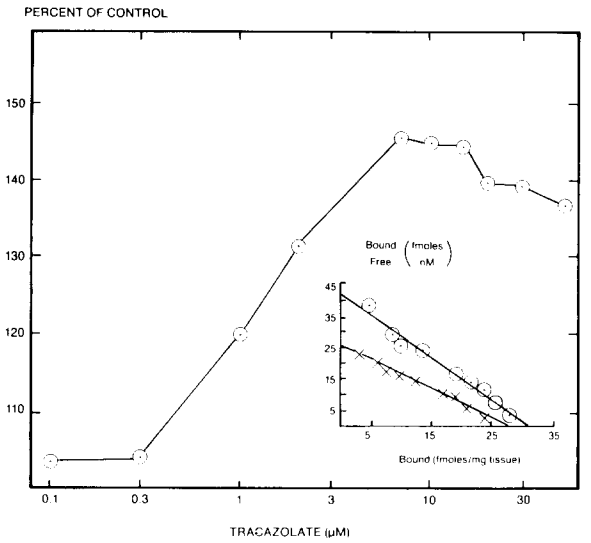

FIG. 5. Effects of tracazolate on [^{3}H] flunitrazepam binding. Insert: Scatchard plot of [^{3}H] flunitrazepam binding in the presence and absence of 10 μM tracazolate. The concentration of flunitrazepam ranged from 0.2 to 9 nM in the presence (O) or absence (X) of 10 μM tracazolate. The binding constants were obtained by unweighted linear regression analysis. The apparent dissociation constants (Kd) with and without tracazolate were 0.7 and 1.2 nM, respectively, with the number of sites (Bmax) being 31 and 28 fmoles per mg tissue, respectively. The difference in the number of sites is not significant.

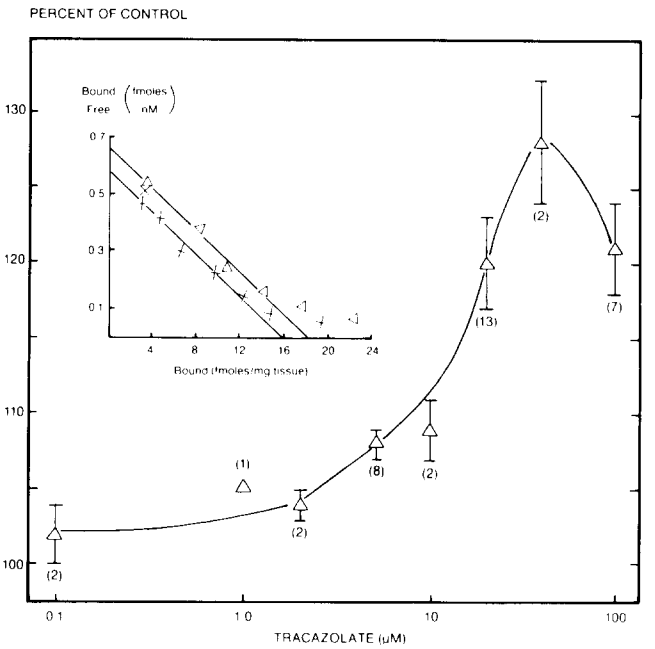

FIG. 6. Enhancement of GABA binding by tracazolate. The figure shows the mean percentage of control for separate experiments (±S.E.M.) or, when only two experiments were averaged, one-half the range). Insert: Scatchard plot of the effect of tracazolate on [^{3}H] GABA binding. The GABA concentration ranged from 6 to 300 nM. The line is a linear least squares fit to the points from 6 to 80 nM GABA. In the presence of 20 μM tracazolate (△), the apparent dissociation constant (Kd) was 26.5 nM and the number of sites (Bmax) was 17.5 fmoles/mg tissue. In the absence of drug (X), these numbers were 26.4 nM and 15.2 fmoles/mg tissue, respectively. The change in Bmax without change in Kd is typical of 5 experiments.

strongly with their potencies in clinical and animal studies as anxiolytics, anticonvulsants and muscle relaxants [5, 16, 20, 26]. Therefore, it was of particular interest to determine the effect of tracazolate on ^{3}H-flunitrazepam binding.

To determine the comparative effects of tracazolate and benzodiazepines, studies were conducted using a modification of the method of Speth and co-workers [26] as described in detail by Meiners and Salama [19]. Unlike the benzodiazepines, tracazolate exhibited a concentration dependent enhancement of ^{3}H-flunitrazepam binding in rat brain cortical membrane fragments (Fig. 5). Scatchard analysis reveals that tracazolate reduced the dissociation constant (K_D) and produced no change in the number of binding sites (B_{max}) [19]. Chloride ion significantly potentiated ^{3}H-FLU binding produced by tracazolate. Similar findings have been reported with two other pyrazolopyridines, cartazolate [2,25] and etazolate [25].

GABA, like pyrazolopyridines, also enhances ^{3}H-benzodiazepine binding due to an affinity change (Wastek *et al.* [29]). In fact, the enhancement of ^{3}H-flunitrazepam binding by tracazolate and GABA are additive even at maximum concentration (100 μM GABA) [19]. This indicates that the enhancement activity of tracazolate and GABA may be occurring at different sites. Interestingly, however, the enhancement produced by GABA and tracazolate was reversed by bicuculline [19]. These data suggest that GABA receptors may play some role in the action of pyrazolopyridines on benzodiazepine binding.

Effect on GABA Binding

Several reports and reviews strongly indicate that the benzodiazepines mediate their pharmacological effects by interacting with a macromolecular complex consisting of a

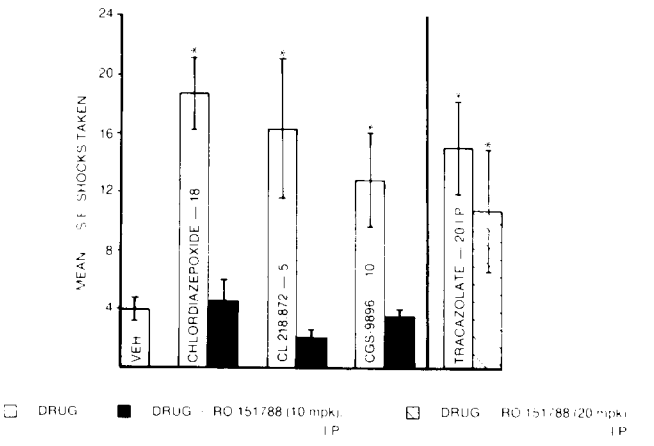

FIG. 7. Mean number of shocks taken in the SSD test following oral treatment (60 min pretest) of selected anxiolytics alone and in the presence of antagonist (30 min pretest). *$p<0.05$ Doses (mg/kg, PO) of each agent follow name in treatment bars.

benzodiazepine receptor, a GABA receptor, and a chloride ionophore [6, 7, 11, 21, 27]. Therefore, studies were conducted to measure the effects of tracazolate and several benzodiazepines on ^{3}H-GABA binding in the rat brain cortical membrane fragments. The details of experimental conditions are discussed elsewhere [19]. As demonstrated in Fig. 6, both tracazolate and benzodiazepines enhance ^{3}H-GABA

binding. However, in general, the effects of tracazolate are somewhat greater in magnitude. In addition, tracazolate enhancement is due to an increase in the number of GABA binding sites with no change in affinity and is chloride dependent [19]. Similarly, cartazolate has been shown to enhance GABA binding [25]. In contrast, benzodiazepine-induced enhancement of GABA binding appears to be mediated through a change in the affinity of the receptor.

EFFECTS OF BENZODIAZEPINE ANTAGONISTS

Benzodiazepine antagonists, RO 15-1788 and CGS 8216, have been reported to bind with high affinities to ^{3}[H]-diazepam binding sites and to antagonize several activities of benzodiazepines (e.g., anticonflict, anticonvulsant, sedation) [3,12]. Therefore, in the present study, a benzodiazepine antagonist (RO-15 1788) was used as a tool in order to determine whether it would antagonize the anticonflict activity of these novel nonbenzodiazepine agents as measured by the SSD test. As shown in Fig. 7 the benzodiazepine antagonist RO 15-1788 significantly attenuated the anticonflict activity of the benzodiazepine displacers. In contrast, the anticonflict activity of tracazolate was not significantly affected even when the higher dose (20 mg/kg, IP) of antagonist was used.

This has also been confirmed neurochemically. Specifically, benzodiazepine antagonists were examined on the enhancement of ^{3}H-GABA binding caused by pyrazolopyridines and benzodiazepines (e.g., diazepam). The results of these studies have shown that the enhancement induced by diazepam was significantly attenuated by the antagonists, whereas tracazolate's effects on ^{3}H GABA binding were unaltered [18].

These data, coupled with our previous findings [24], suggest that the activity of agents that bind to benzodiazepine receptors is reversed by benzodiazepine antagonists, whereas the anticonflict activity of those agents that do not displace benzodiazepines from their receptors are not sensitive to the activity of the benzodiazepine antagonists.

SUMMARY AND CONCLUSIONS

The pyrazolopyridines represent a chemically unique class of anxiolytics . In particular, tracazolate demonstrated activity in preclinical laboratory tests considered to be predictive of anxiolytic activity in man. Tracazolate exhibited anticonvulsant activity in rodents, though its activity is weaker in comparison to benzodiazepines.

The salient feature of tracazolate is that it appears to be a much more selective anxiolytic than the benzodiazepines in that it exhibited therapeutic effects at much lower doses than those at which sedative properties occurred. In addition, unlike benzodiazepines, tracazolate is less likely to potentiate the sedative properties of ethanol or barbiturates in man. Neurochemically, pyrazolopyridines differ from benzodiazepines in that they enhance ^{3}H-flunitrazepam binding rather than displace it from brain sites, indicating that tracazolate may have a novel mechanism of action; e.g., tracazolate may enhance the binding of an endogenous anxiolytic substance. The enhancement induced by tracazolate was due to an increase in affinity and was reversed by a GABA antagonist, bicuculline. Like benzodiazepines, tracazolate enhanced ^{3}H-GABA binding. The enhancement of ^{3}H-GABA binding by pyrazolopyridines and benzodiazepines are additive and reversed by picrotoxin. The actions of pyrazolopyridines may be related to their effects upon a GABA-stimulated chloride ionophore site. Benzodiazepine antagonists reverse neither the anxiolytic properties of enhancers nor their ^{3}H-GABA binding enhancement effects. In summary, pyrazolopyridines appear to represent a novel class of nonsedative anxiolytics which also interact to a lesser extent with alcohol than benzodiazepines.

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