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[^{3}H]-Flunitrazepam Binding in the Presence of β-Phenylethylamine and its Metabolites

THOMAS M. SMITH

Analytical Psychopharmacology Laboratory, The Nathan S. Kline Institute for Psychiatric Research Orangeburg, NY 10962

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SMITH, T. M. *[^{3}H]-Flunitrazepam binding in the presence of β-phenylethylamine and its metabolites.* PHARMACOL BIOCHEM BEHAV **23**(6) 965–967, 1985.—It has recently been reported that the concentration of β-phenylethylamine (PEA) was elevated in the plasma of an individual experiencing convulsions because of an overdose of tranylcypromine. Also, high concentrations of PEA, injected into mice, were reported to induce convulsions. This convulsive effect was prevented by pretreatment with the benzodiazepines diazepam and chlordiazepoxide. In this study, PEA in concentrations from 0.5 to 100 μM failed to alter the binding of [^{3}H]-flunitrazepam ([^{3}H]-FLU) in membrane preparations from mouse rostral forebrain. The metabolites of PEA: phenylacetic acid, phenylethanolamine, octopamine and tyramine, also failed to affect [^{3}H]-FLU binding. This suggests that although there are substances that act as convulsants by interacting with the benzodiazepine receptor sites, the convulsant effect of PEA and its metabolites is mediated elsewhere.

[^{3}H]-Flunitrazepam binding β-Phenylethylamine Phenylacetic acid Phenylethanolamine Tyramine Octopamine

β-PHENYLETHYLAMINE (PEA) is found in low concentrations in mammalian brain [11, 24, 27]. Within the brain, its distribution varies, with the striatum and hypothalamus containing the highest levels [16,27]. This trace amine is a substrate for type B monoamine oxidase [29], has a rapid turnover rate [28] and has a variety of pharmacological actions. It possesses sympathomimetic activity [2,17], causes the release of serotonin [18], norepinephrine [15], dopamine [12] and increases the level of homovanillic acid [1]. Several behavioral characteristics are observed after the administration of this trace amine. It possesses anorectic properties [9], produces stereotyped behavior [23] and gives rise to a conspicuous hyperactivity syndrome [21].

One of the effects of an overdose of a monoamine oxidase inhibitor is the production of convulsions [3]. This effect may occur after a delay of 6 to 12 hours, due to the time it takes for metabolites such as PEA to accumulate [30]. High doses of PEA injected into mice result in the production of convulsions which are prevented by pretreatment with diazepam or chlordiazepoxide [8]. There are compounds which have an affinity for the benzodiazepine receptor complex and lower the seizure threshold and may cause convulsions which are reversed by benzodiazepines such as diazepam. Examples are methyl β-carboline-3-carboxylate (β-CCM) [5], ethyl-β-carboline-3-carboxylate (β-CCE) [22] and 6,7-dimethoxy-4-ethyl-β-carboline-3-carboxylate (DMCM) [6]. Thus it was of interest to determine if the convulsive effect of acute high levels of PEA is due to a direct action on the benzodiazepine receptor. Because of the rapid metabolism of PEA, its available metabolites were also tested.

METHOD

The experimental procedure has been described in detail previously [25]. Briefly, the rostral forebrain of female Swiss Webster mice (35 to 40 g) was separated from the rest of the brain, on ice, after decapitating the animals. The tissue was homogenized in 9 volumes of ice-cold deionized water (Millipore Corp.) with a glass-teflon homogenizer. Centrifugation of the homogenate was at 2000 g for 5 minutes at 4°C. After discarding the pellet the supernatant was centrifuged at 30,000 g for 30 minutes at 4°C. The resulting crude P_2 pellet was resuspended in ice-cold deionized water at five times the original wet weight of the tissue and frozen in 1.0 ml aliquots at −70°C for future use.

Substances making up the incubation mixture were added while the tubes were in an ice bath. The thawed crude P_2 preparation was diluted 50 fold in ice-cold deionized water and dispersed with a glass-teflon homogenizer. One milliliter aliquots of the diluted P_2 suspension were added to the incubation tubes along with, in final concentrations, 50 mM Tris HCl, pH 7.5, 0.2 nM [^{3}H]-FLU and 0.5 to 100 μM of the test compound to a final volume of 2.0 ml. Each determination was done in triplicate. Non-specific binding was determined in the presence of 1 μM clonazepam. The specific binding represented about 95% of the total binding. Upon completion of the additions, the tubes were shaken and then placed in a 37°C water bath for 30 minutes, then in an ice bath for one hour. Incubation was terminated by filtration through Whatman GF/A glass fiber filters on a Millipore 12 sample filtration manifold followed by three washes with 5.0 ml of 50

TABLE 1

EFFECT OF PEA AND ITS METABOLITES ON [^{3}H]-FLU BINDING IN CRUDE P_2 PREPARATIONS FROM MOUSE FOREBRAINS*

Compound	n	Concentrations (μM) 0.5	1	2	5	10	20	50	100
β-Phenylethylamine	5	102 ± 0.2	102 ± 0.2	102 ± 0.3	100 ± 0.4	100 ± 0.2	100 ± 0.2	100 ± 0.3	100 ± 0.2
Phenylacetic acid	4	100 ± 0.2	99 ± 0.2	97 ± 0.8	98 ± 1.0	96 ± 0.2	96 ± 0.6	95 ± 0.7	95 ± 0.2
Phenylethanolamine	4	99 ± 0.2	96 ± 0.6	95 ± 0.2	95 ± 0.2	94 ± 0.6	94 ± 0.2	94 ± 0.9	94 ± 0.2
Octopamine	4	98 ± 0.5	96 ± 0.6	96 ± 0.2	96 ± 0.2	95 ± 0.8	95 ± 0.2	95 ± 0.9	94 ± 1.0
Tryamine	4	101 ± 0.9	100 ± 1.1	101 ± 1.2	100 ± 0.9	100 ± 1.2	97 ± 0.4	97 ± 1.2	96 ± 1.4

Values are the mean (% of control) ±standard error of the mean.

Control value, utilizing 0.2 nM of the ligand in the standard assay containing 3 mg wet weight of tissue, is 2100 cpm at a counting efficiency of 45%.

*None of the values in the table were significantly different from control using the Wilcoxon Rank Sum Test.

mM Tris HCl pH 7.5 buffer. The filters were then placed into plastic scintillation vials (Walter Sarstedt) with 5.0 ml Liquiscint (National Diagnostic) and counted for tritium in a Packard 300C Tricarb liquid scintillation counter.

[^{3}H]-FLU was purchased from New England Nuclear (specific activity 79 to 83 Ci per millimol). PEA, phenylethanolamine, octopamine, tyramine, pargyline and Tris HCl (Trizma Base) were purchased from Sigma while phenylacetic acid was obtained from Aldrich.

RESULTS AND DISCUSSION

It is known that the clinically used benzodiazepines exert their anticonvulsant and anticonflict actions by interacting with the benzodiazepine receptor complex [26]. However, there are recent reports stating that several non-benzodiazepines have the ability to interact with the benzodiazepine binding sites without resulting in an anticonvulsant or anticonflict action. For example, the prazoloquinoline CGS-8216 is a potent benzodiazepine and barbiturate antagonist [7], the imidazodiazepine RO 15-1788 is a benzodiazepine antagonist [14], ethyl β-carboline-3-carboxylate (β-CCE) is a proconvulsant [4], DMCM is a convulsant in mice and rats [6], and methyl β-carboline-3-carboxylate (β-CCM) produces convulsions in mice [5].

The injection of high doses (150 to 200 mg/kg) of PEA into mice produced convulsions which were antagonized by pretreatment with either chlordiazepoxide or diazepam [8] or by increasing GABA levels in brain via GABA transaminase inhibitors [16]. Pretreatment with the benzodiazepines would in effect occupy the benzodiazepine receptor sites, leading to an enhancement of GABA-mediated chloride channel conductance [13]. The occupation of the benzodiazepine receptor site by a convulsant such as DMCM is proposed to result in a reduction in the GABA-mediated chloride channel conductance, which would result in the production of seizures [6].

To determine if the mechanism of action of the PEA-induced convulsions involves the benzodiazepine binding site, this substance was tested, *in vitro*, for its ability to displace [^{3}H]-FLU from its receptor sites in a membrane preparation from the forebrain of mice.

Table 1 shows that PEA in concentrations ranging from 0.5 to 100 μM did not displace [^{3}H]-FLU from its binding sites in this preparation. If the tissue preparation contained monoamine oxidase activity, the test substances would be metabolized before they had a chance to interact with the receptor sites. Therefore, in initial experiments, the crude P_2 preparation was preincubated for 15 minutes at 37°C with pargyline (10 μM final concentration). There was no difference (data not shown) between those assays pretreated with pargyline and those with no pargyline pretreatment.

TABLE 2

EFFECT OF PEA AND ITS METABOLITES ON [^{3}H]-FLU BINDING IN CRUDE P_2 PREPARATIONS FROM MICE UNDERGOING PEA-INDUCED SEIZURES*

Compound	% Control [^{3}H]-FLU binding Saline (n=4)	PEA 200 mg/kg (n=4)
β-Phenylethylamine	100 ± 0.2	100 ± 0.2
Phenylacetic acid	97 ± 0.2	100 ± 0.2
Phenylethanolamine	97 ± 0.2	101 ± 0.2
Octopamine	98 ± 0.4	99 ± 0.2
Tyramine	99 ± 0.4	100 ± 0.4

Values are the mean (% control) ±S.E.M. of 4 mice in each treatment group. All compounds were assayed at a final concentration of 100 μM.

All assay conditions are as in Table 1.

*None of the values in the table were significantly different from control using the Wilcoxon Rank Sum Test.

The metabolism of PEA results in the formation of phenylacetic acid, phenylethanolamine, tryamine and octopamine [28].

Table 1 shows that none of the four metabolites tested inhibited the binding of [^{3}H]-FLU by more than 6% at the highest concentrations used. Thus the metabolites of PEA, like the parent compound, do not have much of a direct effect on the FLU binding site.

During the time required for convulsions to appear in mice after the administration of PEA (<20 minutes), it is possible that changes in the [^{3}H]-FLU binding site may have occured that would effect the affinity of PEA or its metabolites for the binding site. Thus mice were injected (IP) with PEA at a dose of 200 mg/kg which was reported to induce seizures 95% of the time [10]. During the seizure episode,

the mice were decapitated and processed as described above. Control mice were injected with the saline vehicle and sacrificed at the same time the treated mice were.

Table 2 shows that there is no significant difference in the affinity of PEA or its metabolites for the [³H]-FLU binding site in membranes prepared from saline treated mice or those from mice which were undergoing PEA-induced seizures.

In conclusion, although there are compounds that are non-benzodiazepines which are capable of interacting with the benzodiazepine binding sites to produce convulsions, this is not the case for PEA or its metabolites. Thus the mechanism of action of convulsions produced by the acute administration of large doses of PEA in mice and by high plasma PEA concentrations due to tranylcypromine overdose in humans is not a direct interaction at the benzodiazepine binding site.

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