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The Inhibitory Effect of Endogenous Convulsants Quinolinic Acid and Kynurenine on the Pentobarbital Stimulation of [^{3}H]Flunitrazepam Binding

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ZARKOVSKY, A. M. *The inhibitory effect of endogenous convulsants quinolinic acid and kynurenine on the pentobarbital stimulation of [^{3}H]Flunitrazepam binding.* PHARMACOL BIOCHEM BEHAV **24**(5) 1215–1217, 1986.—The metabolites of tryptophan-kynurenines with convulsant action quinolinic acid (QA) and l-kynurenine (l-KYN) antagonized the enhancing effect of pentobarbital (1 mM) on [^{3}H]Flunitrazepam binding. IC_{50} for l-KYN were 35.9±14.8 μM and for QA 31.2±7.2 μM respectively. The inhibitory effect of KYN was stereoselective: IC_{50} of l-isomer was about two fold lower than IC_{50} of racemic form, d,l-KYN. Scatchard analysis revealed that inhibitory effect of QA and l-KYN on [^{3}H]Flunitrazepam binding enhanced by pentobarbital is due to the decrease in affinity of benzodiazepine receptors. On the basis of these data it is proposed that QA and l-KYN possess their convulsant action interacting with barbiturate/picrotoxin sensitive sites of GABA-benzodiazepine-barbiturate complex.

[^{3}H]Flunitrazepam binding Pentobarbital stimulation Picrotoxine L-Kynurenine Quinolinic acid

SUBSTANTIAL evidences suggest that various anticonvulsants and convulsants interact with proposed GABA-benzodiazepine-barbiturate (GBB) supramolecular complex via specific barbiturate/picrotoxin sensitive site [1, 9, 10, 18]. Drugs which directly interact with barbiturate/picrotoxin site in turn may modulate the binding of other ligands to their recognition sites within GBB complex [1, 15, 16, 18, 19]. Among various convulsants the metabolites of tryptophan, kynurenines draw much attention of investigators. Among them quinolinic acid (QA) and l-kynurenine (l-KYN) were most intensively studied last year. These neuroactive substances induce seizures in frogs [5], mice [3, 5, 6, 7] and rats [7,11] after intracerebroventricular administration. In immature rats when the blood-brain barrier is not developed, QA and KYN induce seizures after intraperitoneal injection [4]. Administration of QA into brain structures in rats resulted in neuronal cell loss, and this neuronal damage resembles those induced by exogenous excitotoxins, e.g., kainic acid [13,14]. Recent findings on antagonism of barbiturates to KYN- and QA-induced seizures [12] and on selective synergism of these two endogenous convulsants with picrotoxin among various standard convulsants (Ryzov and Lapin, in press) suggested that the barbiturate/picrotoxin subunit of the GBB can be involved in the central action of KYN and QA. It seemed to be of interest, therefore, to study the action of QA and KYN on the stimulation of [^{3}H]-benzodiazepine binding induced by pentobarbital.

METHOD

[^{3}H]Flunitrazepam ([^{3}H]FNZ, spec. act. 84 Ci/mmol) was purchased from Amersham plc. (UK), picrotoxin, kainic acid, kynurenines: dl-kynurenine, l-kynurenine-SO_4, quinolinic acid, picolinic acid, antranilic acid all were purchased from Sigma (St. Louis, MO). Diazepam and flunitrazepam were a gift of Hoffman La Roche (Switzerland). All other drugs and chemicals were obtained from local commercial sources.

Membrane Preparation and Binding Assay

Fresh brain tissue (without brain stem and cerebellum) from male rats of Wistar strain was homogenized in 10 vol. of ice-cold 0.32 M sucrose in a Potter-Elvehjem homogenizer. The homogenate was centrifuged at 1000 × g for 10 min and the resulting supernatant was recentrifuged at 30,000 × g for 20 min. The pellet from the second homogenization was suspended in 50 vol. of 50 mM TRIS-citrate buffer (pH=7.3), centrifuged at 30,000 × g for 20 min. The pellet then was additionally washed in 50 mM TRIS-citrate buffer three times and stored frozen at −20°C for 24 hr. After thawing membranes were washed at least four times with rehomogenization in the same buffer and finally were resuspended in 100 vol. of buffer. The typical incubations in triplicate contained in a total volume 1.0 ml 0.2–0.24 mg protein of membrane suspension, 1.2 nM [^{3}H]FNZ, 150 mM NaCl, 1 mM

TABLE 1

INHIBITION OF PENTOBARBITAL INDUCED STIMULATION OF [^{3}H]FNZ BINDING BY VARIOUS DRUGS

Drug	IC_{50} (μM)	
Kainic acid	>200	(2)
Quinolinic acid	31.2 ± 7.2	(5)
l-Kynurenine	35.9 ± 14.8	(4)
d,l-Kynurenine	84.5 ± 21.4	(3)
Picolinic acid	99.1 ± 22.5	(3)
Anthranilic acid	>150	(3)
Picrotoxin	6.95 ± 1.25	(5)

For the determination of IC_{50} (concentration producing 50% inhibition) the maximal enhancement of [^{3}H]FNZ binding (by 1 mM pentobarbital) was taken as 100%. The number of determinations is shown in parentheses.

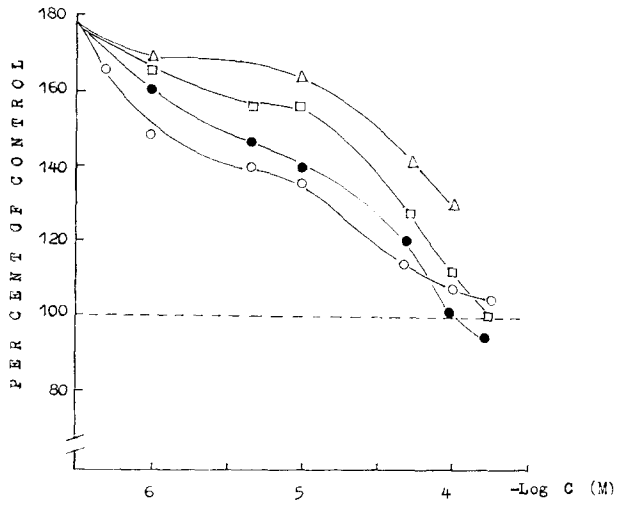

FIG. 1. The inhibitory effect of QA (○), L-KYN (●), d,l-KYN (□) and picolinic acid (△) on the pentobarbital (1 mM) induced stimulation of [^{3}H]FNZ binding in vitro. Abscissa: logarithm of the displacing agent concentration. Ordinate: percent of control. Control was taken as [^{3}H]FNZ binding in absence of pentobarbital (100%).

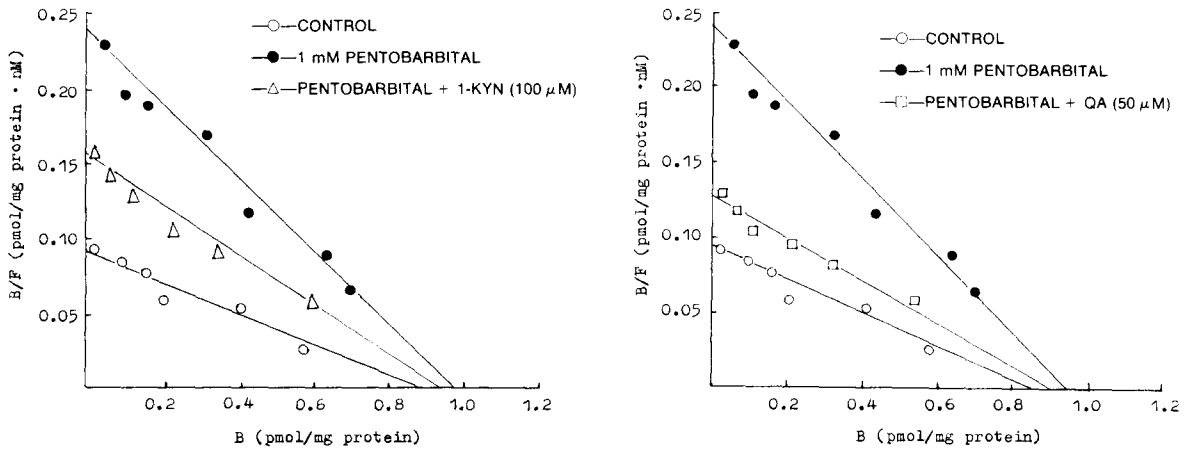

FIG. 2. The Scatchard analysis of [^{3}H]FNZ binding in absence and in presence of pentobarbital (1 mM) and the effect of QA (left) and l-KYN (right) on the pentobarbital-induced stimulation of [^{3}H]FNZ binding.

pentobarbital-Na and various concentrations of the drugs under investigation. The mixture was incubated at 30°C for 30 min and rapidly filtered under vacuum through GF/B filters (Whatman Co.). The filters were rinsed twice with 5 ml of the same buffer and counted in a liquid scintillation counter LS-7500 (Beckman). Specific [^{3}H]FNZ binding was defined as total binding minus binding in the presence of 1 μM flunitrazepam or 5 μM diazepam. The saturation experiments were performed using six concentrations (0.25–16 nM) of [^{3}H]FNZ. Statistics B_{max} (fmoles/mg protein) and K_D (nM) were estimated from Scatchard plots of the binding data using linear regression analysis. IC_{50} for the drugs tested were calculated by probit analysis.

RESULTS

None of the drugs studied affected basal [^{3}H]FNZ binding (data not shown). Stimulation of [^{3}H]FNZ binding by 1 mM pentobarbital varied among experiments from 165.5% to 185.6% (mean=178.3%) above control level (taken as 100%). Picrotoxin effectively inhibited the pentobarbital enhanced [^{3}H]FNZ binding (Table 1). The enhancement of [^{3}H]FNZ binding was antagonized by QA, d,l-KYN and l-KYN-SO_4 (Fig. 1). Among kynurenines the most potent drugs were QA and l-KYN. Moreover, the inhibitory effect of kynurenine was stereoselective: IC_{50} of l-isomer was about two fold lower than IC_{50} of racemic form, d,l-kynurenine (Table 1).

Picolinic acid was much less potent than above mentioned drugs and anthranilic acid was almost inactive. Other excitatory dicarboxylic amino acids: kainic acid and l-glutamic acid taken for comparison (Table 1) did not influence the enhancing effect of pentobarbital on [^{3}H]FNZ binding. Scatchard analysis of the specific [^{3}H]FNZ binding in the presence of pentobarbital showed that the enhancement of binding was due to an increase in affinity without significant changes in the density of the binding sites (Fig. 2). The addition of QA (50 μM) or l-KYN (100 μM) into the incubation mixture produced decrease in affinity of receptors enhanced by pentobarbital (Fig. 2).

DISCUSSION

The principal finding of the present study is that QA and l-KYN effectively antagonize the pentobarbital induced enhancement of [^{3}H]FNZ binding. The effect of l-KYN and QA is due to the decrease in affinity of benzodiazepine receptor enhanced by pentobarbital. Recently it has been shown, that various convulsants including picrotoxin, isopropylbicyclophosphate, benzodiazepine Ro 05-3663, bicuculline antagonize the effect of pentobarbital on [^{3}H]FNZ binding [15, 18, 19]. Based on the data obtained in the present study one may propose that l-KYN and QA possess their convulsant action at least in part via barbiturate/picrotoxin site of GBB supramolecular complex. This suggestion is in agreement with previously obtained data that QA and l-KYN selectively potentiate the convulsant action of picrotoxin and that seizures induced by intracerebroventricular administration of QA and l-KYN are effectively blocked by barbiturates [12]. Moreover, in the present study it is shown that another tryptophan metabolite from the group of kynurenines anthranilic acid which devoid any convulsant activity [5,8] did not antagonize the pentobarbital induced enhancement of [^{3}H]FNZ binding.

It has been proposed in the recent years that there might be an endogenous ligand for barbiturate/picrotoxin receptors in the CNS [18]. Taking into consideration the fact that kynurenines are naturally occuring compounds of the brain one may propose that kynurenines might serve as endogenous ligands or modulators at these receptors. However, the concentration of kynurenines in the brain tissue is much lower than those which induce seizures or inhibit the effect of pentobarbital on [^{3}H]FNZ binding. For this reason a probable role of kynurenines as putative endogenous regulators of barbiturate/picrotoxin receptors under normal conditions requires further investigations.

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