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Differential Effects of α-Methyl-P-Tyrosine and 6-Hydroxydopamine on Pentylenetetrazol Seizures in Mice

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ABED, W. T. *Differential effects of α-methyl-p-tyrosine and 6-hydroxydopamine on pentylenetetrazol seizures in mice.* PHARMACOL BIOCHEM BEHAV **25**(5) 949–952, 1986.—An intraperitoneal injection of the tyrosine hydroxylase inhibitor, α-methyl-p-tyrosine, did not alter the incidence of seizures induced by pentylenetetrazol, but increased the severity and duration of the tonic and clonic phases which resulted in death of some animals. By contrast, pentylenetetrazol seizures' characteristics were significantly changed in response to the intraperitoneal administration of the norepinephrine antagonist, 6-hydroxydopamine, by abolishing the tonic and clonic phases of the seizure. Moreover, α-methyl-p-tyrosine slightly attenuated the protective effect of 6-hydroxydopamine against pentylenetetrazol-induced seizures. Neurochemically, α-methyl-p-tyrosine significantly lowered the brain contents of both norepinephrine and dopamine while 6-hydroxydopamine caused no changes in the brain contents of these amines.

Pentylenetetrazol 6-Hydroxydopamine α-Methyl-p-tyrosine Catecholamines

THERE is increasing evidence to support the hypothesis that brain monoamines play a role in experimentally induced seizures in animals. Thus, increased susceptibility to seizures has been found in rats and mice treated with drugs which deplete brain monoamines [6, 9, 11, 13], while inhibitors of monoamine oxidase or amine precursors such as L-dopa and 5-hydroxytryptophan have been reported to elevate the seizures threshold in these species [9,12].

The present study was designed to determine whether the parenteral administration of α-methyl-p-tyrosine (α-MPT) and 6-hydroxydopamine (6-OHDA) (given individually or concurrently) would change the characteristic manifestations of the seizures induced by pentylenetetrazol (PTZ). The study also involved measurements of brain catecholamine contents prior to and after α-MPT and 6-OHDA administration.

METHOD

Animals

The animals were male albino mice (CFI) supplied by Yarmouk University, weighing 15–25 g at the time of PTZ challenge. The mice were housed in groups of 8–10 and given food and water ad lib. The colony room was maintained on a 12 hr light/dark cycle, and the room temperature was controlled at 23±1°C.

Drugs

The tyrosine hydroxylase inhibitor, α-MPT, was administered intraperitoneally (IP) in normal saline solution (10 ml/kg). The norepinephrine isomer and antimetabolite, 6-OHDA, as the HBr salt was also administered IP in 1% Tween-20 solution (10 ml/kg). Fresh preparations were made for each series of injections. Based on preliminary studies, the standard doses of α-MPT and 6-OHDA were 250 mg/kg and 60 mg/kg respectively. Higher and lower doses were also used.

PTZ Convulsions Test

Pentylenetetrazol was injected IP in normal saline solution (10 ml/kg) one and eight hours after 6-OHDA and α-MPT respectively. A minimal lethal convulsive dose was used: 80 mg/kg. The animals were observed for any changes in behavior prior to and after PTZ injections. Seizures were evaluated on a scale of 0–5 based on the end-point of the seizure sequence: 1—myoclonic jerks; 2—clonic features <10 sec; 3—prolonged or repetitive clonus; 4—brief tonus <5 sec; 5—prolonged tonus lasting 10–50 sec.

Another experiment also involved the administration of PTZ by IP route in a dose of 45 mg/kg to pretreated animals with α-MPT to evaluate the duration and severity of the phases of the seizure. The choice of the dose of PTZ (45 mg/kg) was based on a pilot experiment in which the dose

TABLE 1

EFFECT OF α-MPT ON PTZ SEIZURES IN MICE

Duration of phases of Seizures (sec.)	Placebo	α-MPT
Latent period	54.0 ± 2.8 (10)	60.2 ± 6.2 (10)
Myoclonic jerks	48.6 ± 4.8 (10)	52.5 ± 4.2 (10)
Generalized clonus	8.4 ± 2.3 (10)	23.6 ± 4.8 (10)*
Tonic flexion and extension	Absent	18.3 ± 2.6 (10)
Death	None	4 out of 10

Each value is the mean ± SEM of 10 mice as shown in parentheses.

PTZ dose: 45 mg/kg IP 8 hr after α-MPT (250 mg/kg IP) or placebo.

*$p<0.05$, compared with placebo group.

TABLE 2

EFFECT OF α-MPT OR/AND 6-OHDA ON PTZ SEIZURE IN MICE

		Stage of seizure				
Treatment Drug (s)	Dose (s) mg/kg	Score	Phase I Myoclonic jerks	Phase II Generalized clonus	Phase III Tonic flexion and extension	Death
Placebo	—	5.0 ± 0.0	10/10	10/10	10/10	10/10
α-MPT	250	5.0 ± 0.0	10/10	10/10	10/10	10/10
6-OHDA	40	1.8 ± 1.0†	10/10	2/10	2/10	2/10
α-MPT+6-OHDA	250+40	2.7 ± 1.0*	10/10	3/10	3/10	3/10
6-OHDA	60	1.0 ± 0.0†	10/10	0/10	0/10	0/10
α-MPT+6-OHDA	250+60	1.8 ± 1.0†	10/10	2/10	2/10	2/10

*$p<0.05$; †$p<0.01$; PTZ dose: 80 mg/kg IP 1 and 8 hr after 6-OHDA and α-MPT respectively.

TABLE 3

WHOLE BRAIN CONTENTS OF NE AND DA IN MICE TREATED WITH α-MPT OR/AND 6-OHDA

Treatment	Dose(s) mg/kg	NE (μg/g)	DA (μg/g)
Placebo	—	0.462 ± 0.01 (6)	0.842 ± 0.015 (6)
α-MPT	250	0.178 ± 0.012 (6)*	0.242 ± 0.02 (6)*
6-OHDA	60	0.446 ± 0.02 (6)	0.853 ± 0.02 (6)
α-MPT+6-OHDA	250+60	0.183 ± 0.02 (6)*	0.264 ± 0.018 (6)*

Each value is the mean ± SEM of 6 mice as shown in brackets.

*$p<0.001$; compared with placebo group.

Measurements were determined 1 and 8 hr after IP administration of 6-OHDA and α-MPT respectively.

induced 100% clonic convulsions with the absence of the tonic phase of the seizure.

Measurement of Brain Catecholamines

Brain norepinephrine (NE) and dopamine (DA) were assayed one and eight hours after 6-OHDA and α-MPT respectively, using the spectrophotofluorometric method [5].

Statistical Evaluations

A two-tailed *t*-test for paired data was used for all statistical calculations.

RESULTS

Mice treated with α-MPT (250 mg/kg IP) showed behavioral symptoms similar to those produced by reserpine and their response to external stimuli was reduced. The mice looked less active and less alert than mice treated with vehicle only. They also suffered hypothermia. These behavioral changes were prominent 6–8 hr after administration of α-MPT. Lower doses of α-MPT caused less pronounced changes in behavior. Pretreatment with α-MPT did not alter the incidence of the seizures induced by PTZ (Table 1), but significantly increased the severity and duration of the tonic and clonic phases of the seizures which were followed by the death of some animals. On the other hand, mice treated with 6-OHDA (60 mg/kg IP) exhibited increases in motility, startle response and agitation 45–60 min after administration of 6-OHDA. A dose of 40 mg/kg of 6-OHDA also injected IP caused less marked behavioral changes.

At 1–3 min after injection of a minimal lethal convulsive dose of PTZ (80 mg/kg IP), mice untreated with α-MPT or 6-OHDA showed irregular body movement with hyperkinetic jerks and head twitches (phase I), which lasted 2–5 min. The next stage included brief generalized clonus (2–8 sec) characterized by rhythmic movement of all limbs with the animal lying on its side (phase II). After that, seizures progressed to the tonic flexion and finally into full tonic extension (phase III) with apparent loss of consciousness. Death followed in 20–40 sec. Pretreatment with 6-OHDA produced significant changes in the nature of the seizures by abolishing the tonic and clonic phases and a subsequent reduction in the mortality rate. After 40 mg/kg and 60 mg/kg of 6-OHDA the mean score was reduced from 5.0 ± 0.0 to 1.8 ± 1.0 and 1.0 ± 0.0 respectively and these results were statistically significant ($p<0.01$) as shown in Table 2. By contrast, pretreatment with α-MPT neither affected the incidence of the seizures nor the mean score of the sequence of the phases of the seizures induced by PTZ. Moreover, when α-MPT was given concurrently with 6-OHDA, it caused a slight (though admittedly statistically nonsignificant) reduction in the protective effect of 6-OHDA against PTZ seizures, in which α-MPT allowed some animals to suffer clonic-tonic seizures during which the animals died, which otherwise would have been protected by 6-OHDA (Table 2).

The data presented in Table 3 show that α-MPT caused a highly significant reduction in the brain contents of NE and DA, while 6-OHDA did not affect the brain contents of these catecholamines. Table 3 also shows that 6-OHDA did not affect the lowered brain contents of NE and DA which resulted from α-MPT treatment.

DISCUSSION

The experimental data presented in this paper show that α-MPT not only failed to attenuate the seizures induced by PTZ, but instead increased the severity and duration of the tonic and clonic phases of the seizure, the effects which could have resulted from the depletion of brain catecholamines in response to α-MPT treatment. These results are in agreement with previous reports [2, 6, 9], in which depletion of brain monoamines increased susceptibility to seizures.

With regard to the protective effects of 6-OHDA against PTZ-induced seizures, the data presented in this study support a previous report [1] but differ from those reported by others in which administration of 6-OHDA by the intraventricular route caused an increase in the severity of PTZ-induced and audiogenic seizures [3, 4, 7, 8]. In the latter reports, it is assumed that the amounts of 6-OHDA in the CNS exceeded the concentrations considered necessary for producing degeneration of noradrenergic neurons [1, 14, 15]. The present study in which 6-OHDA was administered IP may have led to gradual infiltration of minimal quantities of unchanged material into the CNS or to penetration by its more stable degradation products without disturbing the whole brain contents of NE and DA. Another possibility also exists, that the observed protective effects of 6-OHDA against PTZ seizures may have occurred via peripheral mediation, since 6-OHDA has been reported to cause initial release of catecholamines from sympathetic nerves before producing neuronal degeneration [10].

The majority of anticonvulsant agents protect from convulsive seizures mainly by reducing the spread of seizure discharges [16] and, like 6-OHDA in this study, abolishes the tonic-clonic phases of the seizure but not the myoclonic jerks.

In conclusion, this study can serve as a model for investigating mechanism(s) involved in propagation of seizures as well as for screening drugs that can reverse the interruption of convulsing discharges.

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