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Anticonvulsant and Convulsant Effects of Organic Solvents[1]

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SILVA-FILHO, A. R., M. L. N. PIRES AND N. SHIOTSUKI. *Anticonvulsant and convulsant effects of organic solvents.* PHARMACOL BIOCHEM BEHAV **41**(1) 79–82, 1992.—Previous studies suggest that organic solvents show anticonvulsant and convulsant effects, respectively at low and high doses. In the present study the first experiment was designed to determine low and high doses of injected acute n-hexane, ethyl acetate and toluene in mice through LD_{50} estimations. In the second experiment, high doses (around LD_{50}) were employed to evaluate the convulsant effects. Finally, the third experiment evaluated the ability of low doses to prevent electroshock- and PTZ-induced convulsions. Results showed that n-hexane increased the severity of the electroshock-induced seizures only at low doses and had no anticonvulsant effects. Ethyl acetate produced generalized clonic seizures and deaths at high doses and was ineffective to prevent electroshock- and PTZ-induced seizures at low doses. Toluene induced forelimb clonus at high doses and protected against electroshock-induced seizures at low doses. Therefore, the biphasic property on convulsant activity seems to be a feature not shared among organic solvents.

Organic solvents Solvents and convulsions Toluene n-Hexane Ethyl acetate

CLINICAL reports have attempted to relate convulsive events and solvent abuse. Grand mal seizure followed by severe uncontrolled status epilepticus was described in a glue sniffer boy (1). Three out of 20 children showing neurological impairment following glue sniffing had convulsions at the admission to a hospital (10). Acute exposure to high concentrations of pure solvents may also produce convulsions (11).

Animal studies have also revealed that convulsions may occur after solvent administration. Generalized tonic-clonic seizures after benzene exposure (100 mg/l of air) and generalized myoclonic attacks after toluene (200 mg/l of air and above) were observed in cats (15). Toluene-exposed rats (2000 ppm) showed a decrease in the convulsion threshold induced by Bemegride (methylethylglutarimide) (20).

On the other hand, a blockade of the electroconvulsive shock (ECS)- and pentylenetetrazol (PTZ)-induced convulsions have been observed after acetone and cyclohexanone administration in rats (12). In addition, low doses of acute toluene and xylene prevented the occurrence of tonic extension and increased the latency to the onset of various convulsant signs in mice receiving 200 mg/kg PTZ intraperitoneally (IP) (23).

The difficulties to explain the results in this field include the variability of seizure tests, solvent types, animal species, doses, exposure times and administration routes employed in the different studies. However, available data point to an anticonvulsant effect of solvents at low doses and a convulsant effect at higher doses.

The present investigation was aimed at evaluating in mice the acute anticonvulsant and convulsant effects of injections of n-hexane, ethyl acetate and toluene employing the same general methods.

METHOD

Subjects

Adult Swiss male mice from our colony, maintained on a 12-h light-dark cycle, in a constant room temperature of 23°C and fed with Purina® chow.

Drugs

Different doses of n-hexane (Merck®), ethyl acetate (Mallinckrodt®) and toluene (Quimica Fina®) diluted in vegetable oil (Polyfarma®) were injected IP and PTZ diluted in isotonic saline was injected subcutaneously (SC).

Experiment I: 50% Lethal Doses (LD_{50}s)

Different groups of mice (15 animals each) received vehicle (0.1 ml/10 g), n-hexane (7, 8 or 9 g/kg), ethyl acetate (0.5, 1.25, 1.5 or 2.0 g/kg) or toluene (2.0, 2.5, 3.0, 3.5 or 4.0 g/kg). The percentage of deaths was recorded 24 h after injec-

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tion. LD_{50}s and potency ratios among the solvents were calculated.

Experiment II: Solvent-Induced Convulsant Effects

Different groups of mice (10 animals each) received vehicle (0.1 ml/10 g), n-hexane (8, 9 or 10 g/kg), ethyl acetate (0.5, 0.75, 1.0, 1.25 or 1.5 g/kg) or toluene (0.5, 1.0, 1.5 or 2.0 g/kg). The animals were isolated and the occurrence of clonus, tonic extension and death were recorded during 60 min. Except for toluene (see the Results section) the doses chosen in the present experiment were around LD_{50} since the literature suggests that convulsions occur preferentially at high concentrations (see introduction).

Control and experimental groups were compared and the 50% convulsant doses (CD_{50}s) were calculated.

Experiment III: Solvent-Induced Anticonvulsant Effects

Groups of 8–10 mice each received vehicle (0.1 ml/10 g), n-hexane (2, 4 or 6 g/kg), ethyl acetate (0.3, 0.45 or 0.6 g/kg) or toluene (0.2, 0.3 or 0.4 g/kg). These doses were chosen based on previous studies suggesting that anticonvulsant effects occur at low doses. Thirty, 60, 90 and 120 min after dosing animals received a transcorneal ECS (15 mA and 0.2 s duration). The time of hindlimbs flexion and extension was measured in seconds immediately after the shock. Mice not exhibiting a tonic extensor seizure after ECS were considered to be protected by the solvent given as pretreatment (19). The hindlimbs extensor/flexor (E/F) ratio (9,18) was also evaluated. A decrease in the E/F ratio is indicative of less severe seizure, while an increased E/F ratio indicates greater severity (18).

In addition, different groups of mice received the same pretreatment described above, but were injected 30 min later with PTZ (100 mg/kg) SC in the back of the neck. Animals were observed for 60 min after PTZ administration. Absence of a single 5-s episode of clonic spasms (a threshold seizure) was defined as protection (13). The occurrence of tonic extension and death were also recorded.

Statistical Analysis

LD_{50}s and potency ratio among solvents (Experiment I), and CD_{50}s (Experiment II) were obtained by the Litchfield and Wilcoxon method (14). Comparisons among control and experimental groups in Experiments II and III were analysed by a Fisher's exact test. In addition, in the Experiment III, one-way analysis of variance (ANOVA) and Duncan's multiple range test were employed to analyse E/F ratio (after ECS) and latencies to the onset of clonus, tonic extension and death (after PTZ).

RESULTS

Experiment I

The LD_{50}s and confidence limits were 1.0 (0.6 to 1.9) g/kg for ethyl acetate, 2.9 (2.7 to 3.2) g/kg for toluene and 9.6 (8.7 to 10.7) g/kg for n-hexane. Figure 1 shows the mortality induced by several doses of each solvent. Potency ratio determination revealed that the three drugs differed significantly in potency (ethyl acetate>toluene>n-hexane) ($p<0.05$).

Experiment II

n-Hexane failed to induce convulsive signs at any of the dosages tested.

Ethyl acetate (0.75 g/kg and above) induced generalized clonic seizures. However, statistical significance was reached only at 1.25 and 1.5 g/kg ($p<0.005$) (Fig. 2). The latency to the onset of the seizures was 7 ± 4 and 4 ± 1 min, respectively for the doses of 1.25 and 1.5 g/kg. CD_{50} was 1.1 (0.9 to 1.2) g/kg. The mortality among mice showing convulsions was 100% at all doses. From the dose of 1.0 g/kg and above several mice showed piloerection, salivation and thoracic spasms together with intermittent mouth openings in the first minutes after injection.

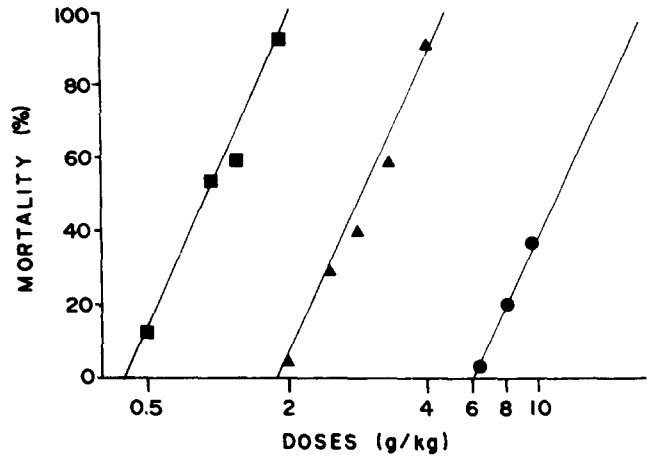

FIG. 1. Dose-response relationship of the mortality within 24 h after acute ethyl acetate (■), toluene (▲) and n-hexane (●) administration (IP) in mice. On the abscissa, the doses are represented in logarithmic scale. Order of potency: ethyl acetate > toluene > n-hexane ($p<0.05$).

Contrasting with n-hexane and ethyl acetate, all doses of toluene tested in the present experiment were lower than LD_{50}. It occurred because the first dose chosen to test convulsant effects (2 g/kg) already showed remarkable convulsive signs. Figure 2 shows that toluene induced forelimb clonus at all doses used, with statistical significance being reached at 1.0 g/kg and above ($p<0.005$). The latency to the onset of forelimb clonus was 9 ± 4, 5 ± 2 and 5 ± 1 min, respectively for the doses of 1.0, 1.5 and 2.0 g/kg. CD_{50} was 0.8 (0.3 to 1.7) g/kg. Furthermore, gross observation revealed increased vibrissae movements in all

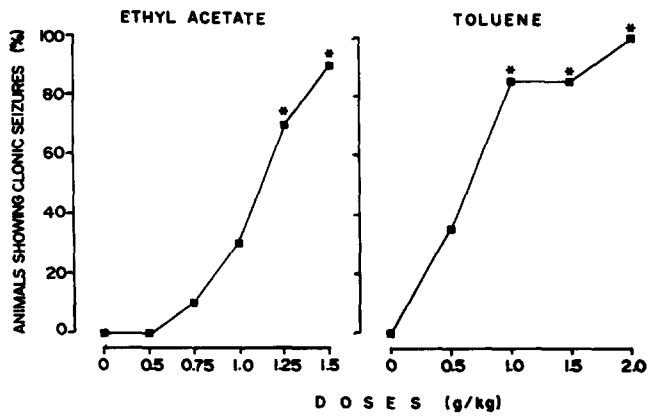

FIG. 2. Clonic seizures induced by acute ethyl acetate and toluene administration (IP) in mice. *Significantly different from control group at $p<0.005$.

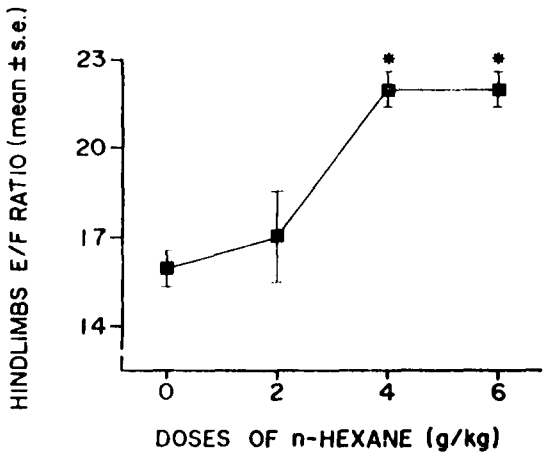

FIG. 3. Increase in the severity of ECS seizures as measured by the hindlimbs extensor/flexor (E/F) ratio 60 min after n-hexane administration. Vertical bars represent standard errors. *Significantly different from controls at $p<0.05$.

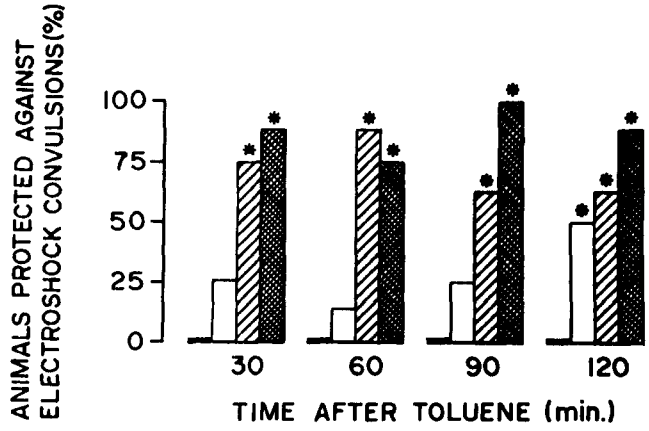

FIG. 4. Protection against convulsion induced by transcorneal electroshock (15 mA, 0.2 s duration) applied at different times after toluene 0.2 (open bar), 0.3 (striped bar), 0.4 (hatched bar) g/kg or vehicle (—) administration (IP) in mice. *Significantly different from control group at $p<0.05$.

animals receiving 1.0, 1.5 and 2.0 g/kg. There were no deaths.

Finally, the three solvents failed to induce tonic seizures at any of the dosages tested.

Experiment III

Animals showed no convulsion signs after the administration of the solvents.

n-Hexane and ethyl acetate failed to prevent tonic extension induced by ECS. While the latter solvent had no effect on the hindlimbs E/F ratio, 4 and 6 g/kg n-hexane increased significantly this parameter 60 min after injections by lengthening tonic extension (Fig. 3), $F(3,36)=11.19$, $p<0.05$. Finally, 0.2 (120 min after injection), 0.3 and 0.4 (at all times tested) g/kg of toluene protected against tonic extension ($p<0.05$) (Fig. 4). Since protection was clearly obtained hindlimbs E/F ratio was not analysed. Clonic seizures after ECS were not blocked by the solvents.

The three solvents tested failed to prevent clonus, tonic seizures and deaths by PTZ. The latency to the onset of these attacks was also unchanged.

DISCUSSION

The hypothetic dose-dependent anticonvulsant and convulsant effects of organic solvents was not sustained except for toluene, as discussed below.

n-Hexane failed to induce both anticonvulsant and convulsant activity by itself. However, it increased the severity of ECS seizures by lengthening tonic extension. Therefore, n-hexane appears to have proconvulsant properties. Interestingly, this proconvulsant effect occurred only at low dosages. Since a proconvulsant effect has been a characteristic ascribed to benzodiazepine antagonists or inverse agonists (3,8), the possibility remains that n-hexane exerts its effects through benzodiazepine receptors. On the other hand, the monovalent anions thiocyanate (SCN-) and perchlorate (ClO_4-) also appear to be proconvulsants. At low doses these agents increase the E/F ratio in mice and rats after ECS, an effect postulated to be exerted by the blockade of K^+-dependent anion transport into glial cells (24). Thus n-hexane could be exhibiting its effects through a mechanism similar to that exhibited by monovalent anions.

Ethyl acetate was much more potent, relative to the other two solvents, in producing acute lethality. It is in general agreement with previous data showing that ethyl acetate possesses greater acute behavioral toxicity than other solvents including toluene (6). At low dosages this solvent failed to prevent PTZ- and ECS-induced seizures. However, it induced generalized clonic seizures and death at high dosages. Additionally, clonic seizures were preceded by autonomic discharges (piloerection and salivation), thoracic spasms and intermittent mouth openings. The occurrence of autonomic discharges followed by seizures has been described as a characteristic effect of cholinomimetic drugs such as carbachol and bethanechol (21). Moreover, the GABA receptor-associated chloride channel blocker picrotoxin (7) also displays such effects (24). Otherwise, the thoracic spasms and intermittent mouth openings suggest the occurrence of hypoxia which, in turn, could lead to seizures and death (22). If so, seizures induced by ethyl acetate could represent a very indirect mechanism of neurotoxicity unrelated to the direct action in the brain. The pharmacological manipulation of drugs acting on cholinergic and GABAergic systems joined with ethyl acetate administration might contribute to elucidate these questions.

Toluene at low doses prevented tonic extension induced by ECS, while high dosages induced forelimb clonus. Therefore, toluene was the sole substance supporting the biphasic property of solvent exposure on convulsant activity. Otherwise, this solvent failed to prevent seizures induced by PTZ. The convulsant effect of toluene was previously observed in mice (17) and cats (15). On the other hand, contrasting with our results, previous data (23) showed that toluene at the same low doses employed here increased the latency to the onset of various PTZ-induced convulsant signs in mice. Although it suggested a benzodiazepine-like effect, the benzodiazepine receptor blockade did not attenuate the anticonvulsant activity of toluene (23). Apparently, the sole factor distinguishing the present study from that abovementioned was the dose of PTZ administered. While those authors administered 200 mg/kg of PTZ, we employed a dose of 100 mg/kg. However, it appears not to be a determinant factor since both dosages induced suprathreshold seizures. We have no explanation for the discrepancy between both studies. In the present work toluene showed effects resembling the anticonvulsant drug diphenylhydantoin (DPH). Typically, DPH protects

animals against ECS- but not PTZ-induced seizures (4,5). Moreover, in spite of conflicting data, it has been observed that toxic doses of DPH induce seizures in mice (2) and humans (16). Finally, the possibility remains that convulsant and anticonvulsant effects of toluene are independent, involving distinct mechanisms of action. The employment of convulsant and anticonvulsant drugs with different mechanisms of action could be an useful pharmacological tool to clarify the biphasic effect of toluene.

In conclusion, present data provide an evidence to refute the biphasic property of solvent exposure on convulsant activity. Although toluene has shown such property it was a feature not shared with the two other organic solvents.

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