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Acute Functional Tolerance to the Motor Impairment Effects of Di-n-Propylacetate[1]

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KULIG, B. M. *Acute functional tolerance to the motor impairment effects of di-n-propylacetate.* PHARMAC. BIOCHEM. BEHAV. 5(5) 511–514, 1976. – Rats were trained to run treadmill fashion along a moving belt to avoid electric shock. After stabilization of performance, the effects of the anticonvulsant di-n-propylacetate (DPA; 100, 200 and 400 mg/kg) on treadmill locomotion were measured. Distrubances in gait and balance were reflected by an increased time off belt in a dose-related manner. In addition, animals showed a progressive improvement over the 3 two-min trials. A second experiment which measured the effects of 300 mg/kg DPA either 5 or 20 min postinjection revelaed that the progressive improvement noted in the first experiment was not due to a diminished drug concentration or to an increased exposure to the drug. Thus, acute functional tolerance to the performance decrement produced by DPA appears to depend upon behavioural processes which enable an animal to overcome the drug-induced functional deficit by practicing the task while in the drug state.

Di-n-propylacetate Functional tolerance Anticonvulsant toxicity Motor co-ordination
Avoidance behavior Treadmill locomotion

THE neurotoxicity of antiepileptic compounds invariably exhibits itself as an impairment of co-ordinated motor function and observational ratings of disturbances of gait and posture are routinely used in the screening of antiepileptics and in the determination of their toxicity [15]. In the clinic, anticonvulsant intoxication with presenting symptoms involving postural and vestibulo-ocular disturbances are encountered [12] and the argument for frequent monitoring of blood levels of antiepileptics has been effectively made [9]. Despite the importance of the effects of anticonvulsants on behavior, both in the determination of toxicity in animal studies and clinically in the treatment of epilepsy *per se*, few attempts have been made to quantitate the effects of antiepileptics on co-ordinated motor function and work on the functional tolerance to these effects, with the exception of the barbiturates, is virtually non-existent.

The anticonvulsant chosen for study in the present experiment was the simple branched-chain fatty acid sodium di-n-propylacetate (DPA). DPA is an effective anticonvulsant in a number of animal models of epilepsy [7] and is used as an antiepileptic in man. Behaviorally, DPA has been shown to interfere with co-ordinated locomotion at high dose levels as measured in a food-motivated task [4]. In addition, DPA has been reported to have no effect on spontaneous motor activity at dose levels below 200 mg/kg and to facilitate the acquisition of learning motivated by aversive stimuli at low dose levels [10].

Although the mechanism of the anticonvulsant or neurotoxic action of DPA is as yet unclear, evidence exists suggesting that both actions might involve changes in gamma-aminobutyric acid (GABA) metabolism [1,4] produced by an interference with the GABA-metabolizing enzymes 4-aminobutyric-2-oxoglutarate transaminse [3] and succinic semialdehyde dehydrogenase [13].

The test chosen to study the effects of DPA on co-ordinated locomotion was a learned complex task which required an animal to remain of a narrow motor-driven belt to avoid an electrified grid. [2]. The apparatus was constructed in such a way that an animal was forced to run treadmill fashion completely on the belt to avoid the shocked grid. Deficits in performance, including impaired co-ordination, loss of balance, or a failure to keep in motion are reflected by an increased time off belt. Efficient treadmill locomotion is readily acquired using this procedure [2] and the test has been shown to provide a stable baseline on which to measure drug effects [2, 5, 6].

The first experiment was designed to assess the dose-dependent effects of DPA and to determine the sensitivity of the moving belt test in the study of the neurotoxic effects of anticonvulsant drugs. The second experiment investigated the nature of the functional tolerance observed at low and moderate dose levels in Experiment 1.

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EXPERIMENT 1

METHOD

Animals

Six naive male Wistar rats (SPF, Organon-originated) weighing 350–400 g were obtained from the animal colony of the Dept. of Pharmacology, Univ. of Leiden. Animals were housed two per cage and received water and Hope Farms lab chow ad lib. Animals were maintained on a 12 hr light-dark cycle (light from 1300 to 0100 hr) and testing was conducted from 1300 to 1700 hr.

Procedures

Rats were trained to avoid electric shock (180 μA, 300 VDC, constant current) by running treadmill fashion along a moving belt (width, 5.6 cm; length, 46 cm; speed, 7 cm/sec) using techniques previously described [2,5]. Each training session consisted of 3 two-min trials spaced 30 sec apart. An animal was considered off the belt if one or more paws touched the grid floor and time off belt was noted by visual observation and recorded by means of a stopwatch.

When animals were performing reliably at 99% avoidance, the effects of saline and three doses of sodium di-n-propylacetate (supplied as a gift of Depakine ® from Labaz B. V.) were measured. Each drug session consisted of 3 two-min trials spaced 30 sec apart and began 15 min following IP administration. On Day 1, animals received a 4 ml/kg IP injection of saline. After the saline control session, the animals were randomly assigned to three groups and on each of three subsequent drug test days were given 100, 200, or 400 mg/kg DPA (4 ml/kg), such that all animals received each dose once in a counterbalanced fashion. On the fifth drug test day saline was again administered to all animals. Drug test sessions were seperated by 3–4 days, except in the case of the second saline test which was carried out 24 hr following the last DPA injection. The animals were tested on the moving belt test daily five days per week.

Immediately after the completion of the third trial on each DPA test day, a 100 μl blood sample was collected from the tail vein. DPA concentration in whole blood was determined by gas chromatographic methods previously described [8] with 2-ethyl-2-methylcapronic acid as the internal standard. Blood analyses, injections, and behavioral testing were carried out without prior knowledge of dose given.

RESULTS

The effects of saline and 100, 200, and 400 mg/kg on moving belt performance are presented in Fig. 1. A treatments × treatments × animals analysis of variance between the first and second saline sessions revealed no significant difference in performance between the two conditions ($F = 1.48$) or in the performance over trials for either saline session ($F = 1.22$) and thus, these data have been combined for graphic presentation. A two-factor mixed analysis with repeated measures on one factor revealed that impairment of moving belt performance was dose-dependent ($F = 13.33$, $p<0.001$). Moreover, there was a significant change in performance over trials ($F = 8.07$, $p<0.005$) and the interaction between dose and trials was also significant ($F = 5.31$, $p<0.001$). Inspection of the figure reveals that performance following 100 and 200 mg/kg DPA was progressively less impaired across the three trials. Although the degree of initial impairment following 100 mg/kg was slight, it did differ from saline levels ($t = 1.82$, $p<0.05$, one-tailed). Performance following 200 mg/kg was markedly improved over the 3 trials, however animals receiving 400 mg/kg showed no functional tolerance to the performance impairment effects of DPA over the three trial session.

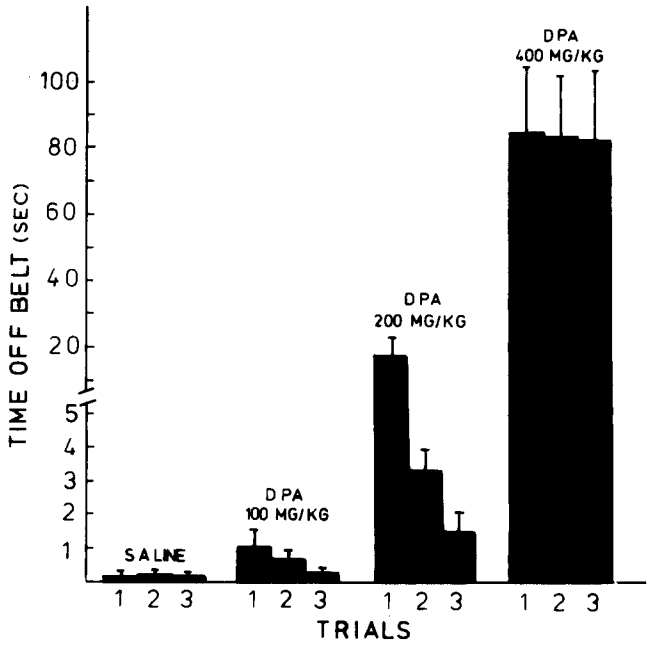

FIG. 1. Dose-response effects of DPA on moving belt performance measured in 3 two-min trials.

EXPERIMENT 2

It was conceivable that the progressive improvement in performance noted at moderate and low doses of DPA was due to changes in DPA concentration within the 7 min test session. However, assuming a steady concentration over the test session. the question still remained as to whether the functional tolerance was due to behavioral factors, i.e. learning to overcome a functional deficit, or to the increased exposure of the central nervous system to the drug from Trial 1 to Trial 3. The second experiment was designed to examine these possibilities.

METHOD

Ten naive male rats of the same strain were maintained and trained as described above. Following stabilization of performance on the moving belt test, the effects of saline and DPA (300 mg/kg) at two different time intervals were measured. Following the first saline control session, rats were randomly divided into 2 groups and on subsequent test days received 300 mg/kg DPA IP and performance on 3 two min trials was measured 5 or 20 min postinjection. As in Experiment 1, each animal received each test condition once, and test sessions were separated by 3 days, except the second saline test which was carried out 24 hr after the last DPA session.

RESULTS

The effects of 300 mg/kg DPA at 5 and 20 min postinjection and the effects of equivalent volumes of saline on moving belt performance are presented in Fig. 2.

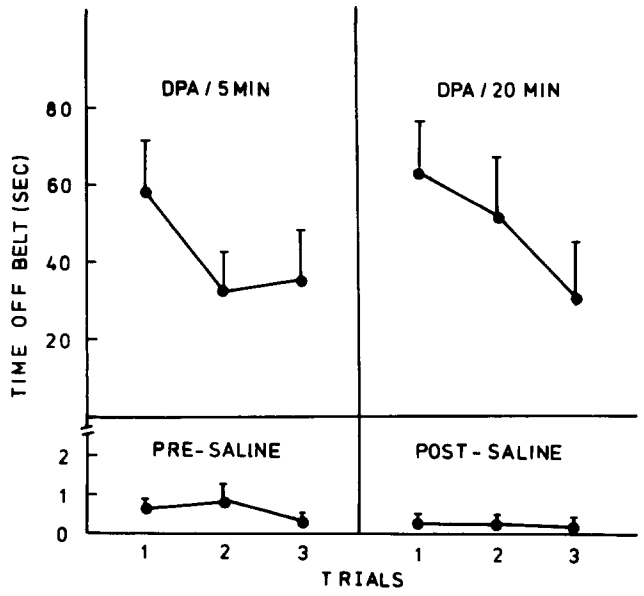

FIG. 2. The effects of DPA on moving belt performance measured in 3 two-min trials either 5 of 20 min postinjection.

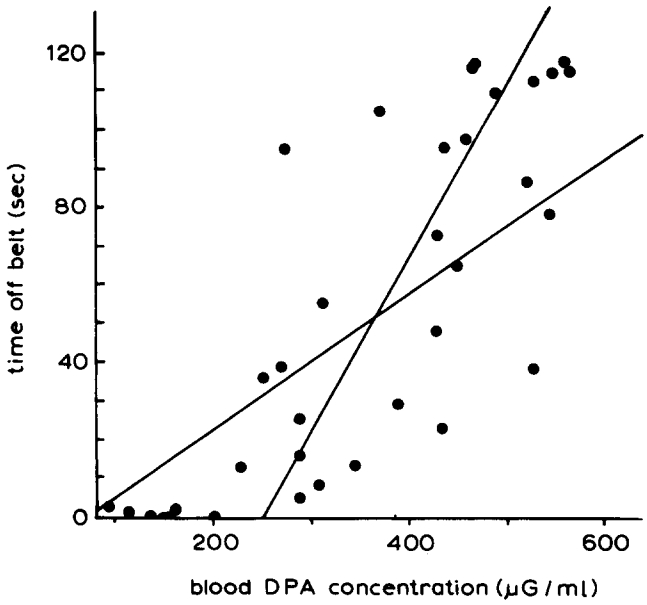

FIG. 3. The relationship between blood DPA levels and performance on Trial 1 in the Moving Belt Test.

Impairment on Trial 1 was equivalent for the 5 and 20 min groups ($F < 1.00$). Moreover, both groups demonstrated a decreased impairment over the three trials ($F = 9.66$, $p<0.001$), and the rate of improvement for the two conditions was significantly different, F (trials x groups) = 1.72. As in Experiment 1, there was no difference between pre- and postsaline performance ($F < 1.00$) and treadmill performance returned to control levels within 24 hr of DPA administration.

The blood concentration of DPA was also examined for the two conditions. DPA concentration (mean ± S.E.M.) for the 5 min group was 368.7 ± 64.5 μg/ml and for the 20 min group, 431.0 ± 41.1. This difference was not satatistically significant ($t = 1.19$).

Finally, to assess the relationship between blood concentration of DPA and the degree of performance impairment the data from both experiments were pooled (Fig. 3). Regression analyses between time off belt on Trial 1 and blood DPA concentration following 100, 200, 300, or 400 mg/kg revealed a significant correlation ($r = .70$, $t = 12.56$, $p< 0.001$). In predicting the performance variable, it was found that: time off belt on Trial 1 = 0.17 x DPA concentration − 11.74; and in predicting the DPA blood concentration, DPA concentration = 2.13 x time off belt on Trial 1 + 254.80.

DISCUSSION

The impairment effects of DPA could be measured in the moving belt test when DPA concentration in whole blood ranged from 100 to 500 mg%. The test was able to differentiate dose-dependent effects well below the anticonvulsant ED50 and below those reported for other tests of changes in motor behavior [4, 7, 10]. Thus, these data suggest that the test may find a valuable application in the general assessment of the neurotoxic effects of antiepileptics.

The qualitative nature of the DPA- induced performance impairments was similar to those reported previously [4]. The DPA-treated rats repeatedly lost their balance, staggering as they ran along the belt. Incoordination and ataxia, particularly of the hind legs was apparent. At the highest dose levels, animals did not keep in motion throughout the trials. Typically, after a series of attempts at moving along the belt, they were forced on to the grid by the baffle at the end of the apparatus and remained with their hind legs on the grid while they continued to move their front paws along the belt treadmill fashion.

With respect to the development of tolerance, it did not appear that prolonged exposure to DPA during a single session contributed to the rate of tolerance development. This finding is in contrast to those reported for ethanol [6]. With ethanol, an increased length of exposure to the drug on an acute basis contributes to a diminished motor impairment effect when animals received a single trial on the moving belt test. When these data are compared to those in the present study and those of Kulig [5] which investigated behavioral processes in the development of acute functional tolerance to ethanol, it appears that different compounds exhibit qualitatively different acute functional tolerance profiles to performance impairment effects. If these differences are shown to be reliable, a question for future research remains as to whether the determination and comparison of such profiles would have any importance with regard to other pharmacological aspects of a given drug (e.g., addiction or abuse potential).

Finally, it must be remembered that efficient performance on the moving belt test requires the integrated functioning of a number of sensory and motor systems to perform a learned avoidance task. It is quite possible that DPA not only impairs motor performance through its effects on GABA-ergic cerebellar pathways [11] as previously suggested [4], but that the drug may also alter sensory and motivational systems or even learning and memory processes themselves which are necessary for the successful execution of learned avoidance responding. The role of GABA in central nervous system function and behavior has not been fully elucidated nor have extensive psychopharmacological studies of the effects of DPA been

carried out. Thus, further studies both at the behavioral and biochemical levels will hopefully shed light on whether DPA-induced performance impairments reflect only changes in co-ordinated locomotion or whether the drug has specific effects on higher-order processes, and moreover, whether such changes involve alterations in the functioning of GABA-ergic neurons.

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