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BRIEF COMMUNICATION

# Effect of DSP4 on Hippocampal Kindling in Rats[1]

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BORTOLOTTO, Z A AND E A CAVALHEIRO *Effect of DSP4 on hippocampal kindling in rats* PHARMACOL BIOCHEM BEHAV **24**(3) 777–779, 1986 —The effects of DSP4, a noradrenergic neurotoxin, on hippocampal kindling were examined in rats The depletion of NA induced by intraperitoneal injection of DSP4 (50 mg/kg) facilitated the rate of hippocampal kindling by reducing the time spent in stage 2 The number of wet-dog shakes was also reduced in experimental animals during kindling stages 1 and 2 compared to control group The results indicate that in DSP4 treated rats there is a more rapid activation of structures involved in the propagation of the local limbic seizure to motor centers responsible for the behavioral manifestation of the seizure, which is due to the NA depletion

DSP4 Noradrenergic neurotoxin Noradrenaline Kindling Hippocampus Epilepsy Seizure

THE role of the brain catecholamines in the pathophysiology of epilepsy has been extensively studied Administration of drugs which deplete catecholamines or destroy terminals containing catecholamines, such as 6-hydroxydopamine (6-OHDA), has been found to facilitate the convulsive response in several models of seizure induction [6, 12, 21] Kindling, originally described by Goddard *et al* [9], has been proved to be a particularly useful model of epilepsy, since it permits a gradual and controlled development of the epileptic condition with no degenerative changes in the tissue surrounding the stimulating electrode [10]

Accumulating evidence indicates that noradrenaline (NA), more than dopamine (DA), may be involved in the development of kindling [1, 5, 6, 20], although NA does not seem to affect the manifestation of seizure once kindling development has been achieved [27]

Recently, N-[2-chloroethyl]-N-ethyl-2-bromobenzyl-amine (DSP4), a nonadrenergic neurotoxin, has been introduced as a tool in the study of NA related functions [2, 13, 23] A systemic injection of DSP4 induces a long-lasting depletion of endogenous NA in several brain regions [11,23] It was, therefore, considered of interest to characterize the effects of DSP4-induced NA depletion on hippocampal kindling

## METHOD

Male Wistar rats, weighing 200–220 g at the time of surgery, were anesthetized with a chloral hydrate/pentobarbitone mixture and were prepared as follows A bipolar electrode constructed of two strands of 100 μm diameter nichrome wires twisted together and insulated except at the cross sections of the tips, was stereotaxically implanted in the right dorsal hippocampus according to the atlas of De Groot [7] (A 4, L 2 5, H +2 8) In addition, three screw electrodes were inserted into the skull, two over the occipital cortex for EEG recording and one in the frontal sinus for grounding One week after surgery the animals were injected with DSP4, 50 mg/kg, intraperitoneally, or with an equal volume of physiological saline Two weeks elapsed between drug treatment and beginning the kindling procedure The stimulations (2 sec train of 60 Hz, biphasic, 1 msec rectangular pulses) were delivered through a constant-current stimulator The intensity was adjusted every 10 min until a short after-discharge (AD) was evoked Then, the animals were stimulated on subsequent days, twice a day (at 0900 and 1700) The intensity was held constant throughout all subsequent procedures The electrical responses were recorded from the stimulating site and from the cortex For each animal, the following criteria were scored duration of AD, number of wet dog shakes (WDS), behavioral stages as defined by Racine [22], number of stimulations necessary to trigger each stage and total kindling rate Two weeks after termination of stimulations,

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TABLE 1
EFFECT OF DSP4 ON HIPPOCAMPAL KINDLING

| Group | Number of ADs to first stage-5 seizure | Duration of each kindling stage (number of ADs) | | | |
|---|---|---|---|---|---|
| | | Stage 1 | Stage 2 | Stage 3 | Stage 4 |
| DSP4 | 25 7 ± 5 1* | 6 4 ± 1 71 | 12 9 ± 3 92* | 2 7 ± 1 48 | 2 8 ± 1 31 |
| Saline | 44 6 ± 3 6 | 6 2 ± 1 55 | 30 6 ± 6 68 | 4 8 ± 4 23 | 2 6 ± 1 26 |

Data are expressed as mean ± SD Each group consisted of 10 animals
*Different from saline group $p<0$ 01 (Student $t$-test)

some of experimental and control animals, randomly chosen, were decapitated for monoamine determinations Brains were quickly removed and dissected on ice into hippocampi and cortices (left and right hemispheres pooled) The tissue was then processed according to Kehr *et al* [14] The remaining animals were deeply anesthetized and perfused through the heart with saline solution followed by 10% formalin solution The brains were then removed and fixed in formalin Paraffin sections were taken in the frontal plane throughout the entire brain and stained with cresyl violet Histological examination showed the loci of electrode tips within the dorsal part of the right hippocampus

## RESULTS

Rats treated with DSP4 in the dose of 50 mg/kg showed a marked depletion of NA in the hippocampus and cortex (18 and 22% of controls, respectively), without major alterations in the concentration of DA, when determined 10 weeks after injection of DSP4 The DSP4-induced NA depletion had no significant effect on the mean threshold for local AD (DSP4—23 8±2 5 $\mu$A, Saline—24 0±5 8 $\mu$A) as well as on the duration of the first primary-site AD (DSP4—30 0±8 8 sec, Saline—29 0±6 6 sec) Conversely, NA depletion did affect the rate of kindling development, with treated animals developing state 5 seizure more rapidly than their controls (Table 1) Calculating and comparing the duration of each kindling stage we could observe, in the experimental group, a reduction in time spent in stage 2 compared to controls, which is fairly well related to the facility of the kindling onset in this group The time spent in other stages was practically the same for both groups of animals (Table 1) As can be observed in Fig 1, DSP4 treated rats showed a reduced number of WDS during stages 1 and 2 compared to their controls

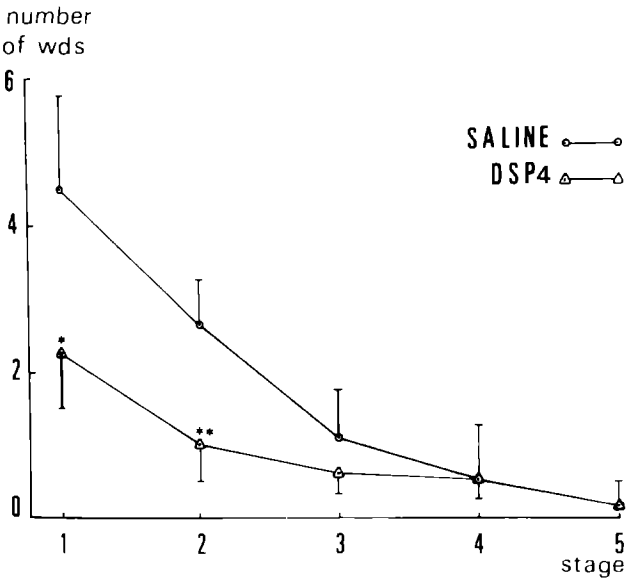


FIG 1 Number of wet-dog shakes during each kindling stage Vertical bars indicate the standard deviation Different from saline group for *$p<0$ 005 and **$p<0$ 001 (Student $t$-test)

## DISCUSSION

The present findings indicate that kindling of the dorsal hippocampus is facilitated by DSP4 The facilitation cannot be related to changes in local suspectibility to seizure since DSP4 and control rats did not differ in the threshold and duration of the first primary-site AD These results are similar to those observed during limbic or neocortical kindling in rats with reduced NA concentrations induced by 6-OHDA or by electrolytic lesions of the locus coeruleus [1, 5, 20] One important observation in this study is the relation between the reduction in the time spent in stage 2 and the facilitation of the kindling onset State 2 can be considered as a period in which there is a gradual activation of the structures engaged in the widespread propagation of epileptiform activity from the primary focus [16] Thus, and as pointed out by McIntyre and Edson [20], the kindling facilitation observed in NA depleted animals seems to be dependent on the rate by which local limbic seizure gains access to or influences the mechanism responsible for the diffusion of the epileptic activity throughout the brain leading to a full motor seizure

Another point of interest is related to the occurrence of wet dog shakes (WDS) during hippocampal kindling in DSP4 treated rats In rats, WDS can occur spontaneously following different sensorial stimulations [3,26], can be induced by different drugs [4, 8, 15, 19], and be elicited after electrical stimulations of specific structures of the brain [24] In relation to epilepsy, we have shown that the number of WDS is progressively diminished when septal [17] or hippocampal [18] stimulations are repeated according to the kindling paradigm Secondary generalization only began when the number of WDS was strongly reduced As to seizures induced by kainic acid [19] or pilocarpine [25], a similar relationship was reported between the occurrence of motor seizures and the reduction of WDS Thus, the reduced number of WDS in DSP4 treated rats during kindling stages 1 and 2 seems to be another index of facility by which local hippocampal AD reaches motor centers involved in the behavioral seizure

In summary, it was observed that depletion of NA induced by DSP4 facilitated the widespread propagation of epileptiform activity and the rate of hippocampal kindling The use of two indices, i e , the time spent in each kindling stage and the negative relationship between kindling susceptibility and elicitation of WDS, seems to be particularly useful when discussing changes in local epileptic changes and generalization process

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