Electroencephalographic study of the effect of neurotoxin DSP-4 in iron model of chronic focal epilepsy

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The effect of the noradrenergic neurotoxin N-(2-chloroethyl)-N-ethyl-2-bromobenzylamine (DSP-4) on electroencephalographic activity (EEG) was studied in the model of chronic focal epilepsy induced by intracortical injection of FeCl$_3$ in the rat. EEG activity was recorded from the epileptogenic focus (ipsilateral and contralateral) in chronic experiments before and after DSP-4 treatment. In some experiments EEG activity was also simultaneously recorded from the cortical epileptogenic focus and locus coeruleus before and after DSP-4 treatment to study the effect of iron-induced seizure activity and of DSP-4 on the locus coeruleus electrical activity. The results showed that DSP-4 aggravated the iron-induced epileptiform activity as well as the locus-coeruleus electrical activity. The data also showed that induction of epilepsy by FeCl$_3$ is accompanied by enhancement of the locus coeruleus electrical activity. Our study demonstrates that DSP-4 intensifies and modifies the epileptic activity in the iron-induced chronic epilepsy model and that the effects of toxin persist for a longer duration.

Iron-induced experimental epilepsy model is of general as well as particular interest because of its similarity to human post-traumatic epilepsy$^{12}$. Thus, it is widely studied for understanding the basic mechanism of human post-traumatic epilepsy$^{3-7}$. Possible mechanism underlying the pathogenesis of epilepsy by iron have been described$^{12}$. Briefly, induction of recurrent epileptic seizure activity in the electrocorticogram is believed to be mediated by membrane lipid peroxidation initiated by the action of reactive oxygen free radicals which are produced during iron-tissue interaction$^{8,9}$. In persons who have suffered head trauma, blood extravasation followed by hemolysis results in the deposition of iron within the neuropil and this iron can produce free radicals leading to peroxidation of membrane lipids. That lipid derangements and membrane lipid peroxidation can alter membrane electrophysiological properties has also been demonstrated$^{10}$.

The noradrenergic influence originating in locus coeruleus (LC) neurones has an inhibitory control over electrographic seizure activity$^{11-14}$. The noradrenergic neurotoxin N-(2-chloroethyl)-N-ethyl-2-bromobenzyl amine (also referred to as DSP-4) selectively acts on and produces selective degeneration of central noradrenergic terminals originating from the LC$^{15,16}$. That the DSP-4 administration lowers norepinephrine levels in several brain regions of rats, mice and cats has been shown in a large number of studies$^{12,13,15-20}$. Furthermore, that DSP-4 is an optimal tool for studying the removal of LC (noradrenergic) influence has also been shown$^{21}$.

Effects of DSP-4 have been studied in a variety of animal models of epilepsy, such as the penicillin epilepsy$^1$, electroshock and kindling seizures$^2$. In these models, because of its norepinephrine-depleting effects, DSP-4 was found to aggravate epileptic seizures. However, in a few other animal models of non-convulsive epilepsy, the toxin-induced norepinephrine depletion was accompanied by attenuation of seizures$^{22,23}$ indicating that the control of seizures may be achieved in different ways in different models. It therefore appears that different animal models of experimental epilepsy may respond differently to DSP-4. The objective of the present study was to examine the effect of DSP-4 on epileptic electroencephalographic activity in the iron-induced epilepsy model of focal epilepsy.

DSP-4 has also been found to affect several cellular electrophysiological properties of locus coeruleus neurones$^{24}$. DSP-4 effects on the EEG activity of the LC have not been studied. In the present study, effects of DSP-4 have been observed on LC EEG and correlated with those on the epileptic activity of the cortical focus.
Materials and Methods

Animals—Adult male Wistar rats (50) weighing 250-300 g were used. Animals were housed individually in plastic cages in an air-conditioned animal house room and maintained on a 12 h light/dark cycle, and were given water and food ad libitum.

Chemicals—The chemicals viz. FeCl₃, urethane and DSP-4 were purchased from Sigma Chemical Co. USA.

Production of iron (FeCl₃) epilepsy and recording of electroencephalographic activity—Iron-induced epileptogenic focus was produced by intracortical injection of FeCl₃ (5 µl 100 mM) into the sensorimotor cortex (1 mm posterior and 2 mm lateral to bregma) of the rat according to the methods described earlier. In control rats, the same volume (5 µl) of saline was injected at the same site. For chronic electrocorticographic (ECoG) recordings, electrodes (Plastic ONE, USA) were implanted stereotaxically on the surface of the frontal-parietal cortex on both ipsilateral and contralateral sites: one at 2 mm lateral to bregma and 1.5 mm deep and second at 3 mm posterior, 2 mm lateral to bregma and 1.5 mm deep (both on ipsilateral and contralateral sides). A reference electrode was implanted over the nasal bone. For recording EEG activity from the locus coeruleus, bipolar electrodes were implanted stereotaxically in this structure (9 mm posterior and 1 mm lateral to bregma and 7.6 mm deep). Recording of electroencephalographic activity was performed, using a Grass Polygraph/EEG, for one and a half-month in conscious unrestrained animals as detailed in our earlier publication.

Animal’s behaviour was carefully observed to check for movement artefacts in the recordings. Epileptiform activity was recorded monopolarly from day 3-5 after surgery over a period of one and a half-month from epileptogenic foci and locus coeruleus daily. Each recording session lasted for 2-4 hr without interruption. Occurrence of the epileptiform EEG activity during periods of awake immobility (quiet-wakefulness) were taken into account to avoid contamination with movement and other artefacts.

Effect of DSP-4 on epileptic activity in the cortical epileptogenic focus—Experimental animals were given a single intraperitoneal injection of DSP-4 (50 mg/kg) in 0.5 ml saline according to the method of Culic. After 2 weeks they were made epileptic by intracortical injection of FeCl₃, and were also implanted with cortical electrodes over ipsilateral and contralateral cortices. Control animals were intraperitoneally injected with saline instead of DSP-4. Two weeks thereafter they were also made epileptic by intracortical injection of FeCl₃ and their ECoG activity was recorded as in the case of experimental animals.

Non-epileptic control animals in which intracortical injection of saline was given in place of FeCl₃ were also implanted with electrodes for recording ECoG activity. Electroencephalographic recordings were performed in experimental and control animals as described above after allowing 3-4 days for recovery from surgery.

Effect of DSP-4 on LC EEG—Experimental animals made epileptic by intracortical injection of FeCl₃ were implanted with cortical electrodes as well as intracerebral electrodes for recording EEG activity from the locus-coeruleus. One week thereafter they were given a single intraperitoneal injection of DSP-4 (dose as above). ECoG and LC EEG recordings from these animals were collected by recording daily on weekdays.

Control animals were made epileptic by intracortical injection of FeCl₃ and were implanted with electrodes for recording ECoG and LC EEG activity. One week thereafter they were given a single intraperitoneal injection of saline in place of DSP-4. EEG recordings from the ipsilateral epileptogenic focus and LC were performed in control and experimental animals for over a period of one and a half-month by recording daily on weekdays.

ECoG and LC EEG activity recordings were also obtained from non-epileptic control animals in which intracortical injection of saline was given in place of FeCl₃. These served as control for epileptic activity recorded in the animals of the just proceeding two groups.

For one experiment each of the above groups contained five animals. The experiment was repeated twice. The electrophysiological recordings were thus actually collected from ten animals in each group. The location of electrodes in LC was histologically verified after the experiments using standard procedures.

To obtain a quantitative measure of the DSP-4-induced effect on the epileptic activity of the ipsilateral cortical focus and locus coeruleus, the cumulative epileptic EEG seizure duration for each hour was measured during the period of recording as described by Lannes et al. and de Vasconcelos.
Statistical comparisons between the epileptic EEG seizure duration from control and experimental animals at the corresponding time points were made by Two way ANOVA with repeated measures. Newman-Keuls post-hoc and Student’s t-test were performed to determine differences between group means.

Results

All the animals, which were intracortically injected with FeCl3, developed epileptic EEG activity. The iron-injected rats showed spontaneous recurrent epileptiform discharges consisting of bursts, spikes and multiple spike wave complexes. The time course of the build-up and progression of epileptic EEG features were consistent with previous reports. Behavioural changes associated with epileptic electric activity were also similar to those described previously. Iron-induced epileptic rats do not show violent motor convulsions. Control rats injected intracortically with saline did not show epileptiform activity in their ECoGs (Fig 1A, 3 A).

Effects of DSP-4 on epileptic activity of the cortical focus and locus coeruleus in FeCl3-induced epileptic rats—Animals tolerated the DSP-4 well and there was no mortality. In iron-induced epileptic rats, DSP-4 induced a marked potentiation of the appearance and duration of electroencephalographic seizure activity as observed by increased epileptiform activity (Fig. 2). This was observed in both the cortical epileptogenic focus and in the locus coeruleus (Fig. 4). Comparison of the electroencephalographic recordings obtained from control animals (saline treated iron-induced epileptic animals) (Fig. 1 B-E and Fig. 3 B-F) and those obtained from DSP-4 administered animals, for example (Figs 2 and 4), would clearly show that in the latter the epileptiform activity was highly potentiated and intensified as observed by increased amplitude and higher number of multispikes bursts. In epileptic rats, DSP-4 induced a significant increase in the total epileptic seizure duration both in the cortical epileptogenic focus (Fig. 5) (ANOVA F 3,32 = 862.1, P>0.01) and the locus coeruleus (Fig. 6) (ANOVA F 3,32 = 1303.27, P>0.01) as compared to controls.

Effect of FeCl3-induced seizure activity on LC EEG activity—Fig 3 (B-F) shows examples of EEG recordings from the cortical epileptogenic focus and the LC obtained at different time points following the intracortical injection of FeCl3. The appearance and duration of the cortical epileptiform discharges increased progressively after the intracortical injection of FeCl3. It is also apparent from the recordings that bursts of spike-wave discharges (epileptiform activity) appeared in the locus-coeruleus synchronously with those in the cortical epileptogenic focus and increased progressively and concomitantly with those in the cortical focus.

Discussion

In our colony of rats we have come across no cases which have shown spontaneous spike and wave discharges as are known to occur in genetically epileptic or epilepsy prone rats. Therefore, the...
epileptic activity seen in our FeCl₃-induced epileptic rats was due to the compound of iron.

Our results demonstrate that DSP-4 aggravates the electrographic epileptic activity in the FeCl₃-induced epilepsy model. The increase in cortical epileptic activity of the FeCl₃-induced chronic epileptogenic focus observed in the present study is similar to the DSP-4-induced increase found in the penicillin-induced acute model of epilepsy²¹. Exacerbation of epileptic activity can result from a decrease in the cortical noradrenergic activity due to loss of cortical noradrenergic terminals originating from the locus

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**Fig. 2**—Representative electroencephalographic seizure recordings from DSP-4 treated iron-induced epileptic rats showing the DSP-4 induced enhancement of epileptiform ECoG activity at post-iron-injection days. In this animal, DSP-4 was injected 2 weeks before the intra-cortical injection of FeCl₃ for production of epileptogenic focus. Compare with various daytime points in Fig. 1. 0-Control (only DSP-4 no FeCl₃) At days 5, 10 and 15 the epileptic activity is much higher in comparison with that at the corresponding time points in Fig. 1. At day 20 the activity is already higher than that at day 28 in Fig. 1.
coeruleus13,14. It is known that the DSP-4 causes degeneration of the central noradrenergic terminals with consequent depletion of norepinephrine (NE) levels15, which render the animal more susceptible to seizure producing stimuli14. Furthermore, it is also of interest that DSP-4 administration does not significantly influence 5-HT14,19 and dopamine levels12,17,19. Therefore, exacerbation of epileptic activity observed in the present study can be considered to be due to depletion of NE levels by DSP-4 indicating the involvement of noradrenergic mechanism in the FeCl3-induced model of epilepsy. In one study 32 on the involvement of noradrenergic mechanism in FeCl3 induced epilepsy model, it was found that changes in α and β-adrenergic receptor activities were related to epileptic EEG activity. These data exclusively derived from observations on the cerebral cortex were interpreted to indicate involvement of noradrenergic mechanisms in the FeCl3 induced epilepsy.

Fig.3—Effect of rotor speed and opening roller speed on percentage of leading hooks [rotor diameter: (a) 36 mm, (b) 46 mm, and (c) 56 mm]
DSP-4 administration to non-epileptic rats does not induce electrical\textsuperscript{12,21} or behavioural seizures\textsuperscript{13}. DSP-4 shows only seizure-exacerbating effects in epileptic models\textsuperscript{12} by lowering threshold for seizure induction\textsuperscript{13}. The seizure-aggravating effects of DSP-4 could be due to norepinephrine depletion\textsuperscript{13} because the latter is known to modulate cortical excitability\textsuperscript{13}. Therefore, the present observations on FeCl\textsubscript{3}-induced epilepsy model support the seizure-exacerbating role of DSP-4. In genetically spontaneous generalised non-convulsive epilepsy rats, the DSP-4 effect on epileptic electric activity was found to last for less than 10 days\textsuperscript{12}. In our study of FeCl\textsubscript{3}-induced chronic epilepsy in rats, the DSP-4 effects were more persistent as the increased epileptic activity continued to last for more than 20 days.

![Figure 4](image-url)

**Fig. 4**—Effect of rotor speed and opening roller speed on percentage of middle leading hooks [rotor diameter: (a) 36 mm, (b) 46 mm, and (c) 56 mm]
Fig. 5—Effect of DSP-4 on epileptic seizure duration in ECoGs of ipsilateral epileptogenic focus of iron-induced ipsilateral epileptogenic focus. Each data point represents mean ± SD of values from five animals. Control iron induced epileptic rats; DSP-4: iron-induced epileptic rats in which DSP-4 had been injected i.p. two weeks before iron injection; Comparison of the individual data points of the upper curve with the corresponding ones of the lower curve would show significant increase in epileptic discharge duration in DSP-4 treated animals at days 8, 10, 15 and 20. At day 28 there was increased seizure discharge duration in both but the amplitude was higher in DSP-4 treated rats.

Pentylenetetrazol and pilocarpine-induced seizures are the examples of seizures in which LC activity has been found to increase. The present data showed that, in the FeCl3-induced cortical seizure model also the LC activity was increased as observed by the appearance of high voltage fast activity with spike wave like complexes. In general LC neurones display changes in their activity with behavioural states. Therefore, in the epileptic state their activity is likely to change. Furthermore, enhancement of the LC neuronal activity in turn may induce an increase in the cortical EEG. Given the close temporal relationship between the iron-induced increase in cortical epileptiform activity and LC EEG activity, it is likely that the two structures influence each other.

Concerning the effects of DSP-4 on LC EEG activity the present data showed that the drug increased the LC EEG activity in FeCl3-induced epileptic rats. Previous studies have only shown that DSP-4 produced a reduction in the action potential duration in LC neurones. Our data thus additionally showed that DSP-4 increased the LC EEG activity. Moderate doses of DSP-4 have been found to cause acute loss of locus coeruleus efferents (axon terminals). However, this loss may be followed by recovery and regeneration of noradrenergic fibres although after a long interval. Higher doses of DSP-4 have been reported to induce neurone loss in locus coeruleus causing substantial decreases in the locus coeruleus cell number. Although we have not studied cyto-histological alterations in LC, the increase in LC activity in DSP-4 treated rats may reflect a compensatory activity from remaining NE neurones themselves.

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References


