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(^{3}H) Spiroperidol binding decreases in brains of rats infected with Venezuelan equine encephalomyelitis virus[1]

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Summary. After i.p. inoculation with the Guajira strain of Venezuelan equine encephalomyelitis virus a significant decrease in the density of (^{3}H) spiroperidol binding sites in the striatum, midbrain and frontal cortex was observed. No changes in the affinity of the receptors could be demonstrated. This finding is compatible with neuronal degeneration caused by the viral infection.

We have found that different central aminergic systems are affected by infection with the Venezuelan equine encephalomyelitis (VEE) virus. A significant decrease in the activities of the enzymes glutamate decarboxylase[2], tyrosine hydroxylase[3], and choline acetyltransferase[4] has been observed in several brain regions of mice and rats infected with VEE virus. However, the receptor components of these aminergic transmitter systems have not been studied in animals infected with VEE virus.

(^{3}H) Spiroperidol is at present the most commonly used dopamine antagonist radioligand and it has been widely employed to detect changes in striatal dopamine receptors in response to a variety of conditions. It has recently been shown that both (^{3}H) spiroperidol and (^{3}H) haloperidol bind to the same striatal receptors[5] which seem to be approximately evenly distributed among striatal interneurons and corticostriatal glutamate-containing neurons[6]. It has been reported that in the striatum, (^{3}H) spiroperidol binds mainly to dopamine (D_2) receptors, though in parts of the brain such as hippocampus and frontal cortex, it is known to bind with high affinity to serotonin-related sites also[7]. From studies with an in vivo (^{3}H) spiroperidol radioreceptor assay it has been concluded that (^{3}H) spiroperidol binds mainly to dopamine receptors in hippocampus as well as in striatum, whereas both serotonin and dopamine receptors are labeled in frontal cortex[8]. In order to get a better insight into the pathogenesis of this viral disease, we therefore analyzed the binding of (^{3}H) spiroperidol to its brain receptors, which seem to be located in pre and post synaptic membrane structures that have the characteristics of plasma membranes[9]. We now report evidence that the density of receptor sites (B_{max}) is reduced in VEE infected rats without alterations in the affinity of the ligand for its binding sites.

Sprague-Dawley male rats, weighing 200–300 g were inoculated i.p. with 0.3 ml of a suspension containing 100 LD_{50} of the Guajira strain of VEE virus in 0.4% bovine albumin borate-buffered saline solution (BABS), pH 7.4. To control animals we administered equivalent volumes of BABS. They were killed by decapitation simultaneously with the diseased animals, 6–8 days after the inoculation, when the latter presented signs of encephalitis. The brain was quickly removed and the striatum, frontal cortex, and midbrain were dissected out at 4 °C following the technique of Glowinski and Iversen[10], and stored at −80 °C until analyzed. Each brain region was homogenized and the incubation performed according to a previously reported procedure[11]. The concentrations of (^{3}H) spiroperidol used were: 0.10, 0.25, 0.375, 0.50, 1.00, 1.50, 2.00

Binding parameters of (^{3}H) spiroperidol in the brains of VEE infected rats

Brain regions	N*	B_{max} (fmol/mg protein) Control	Infected	K_d (nM) Control	Infected
Striatum	12	474.3 ± 40.6	342.0 ± 23.6**	0.69 ± 0.06	0.85 ± 0.10
Midbrain	7	142.5 ± 12.3	109.1 ± 8.1**	2.80 ± 0.40	2.37 ± 0.15
Frontal cortex	6	197.2 ± 14.3	110.0 ± 32.1**	1.35 ± 0.39	0.82 ± 0.30

Results represent the means and SE. * Number of assays, ** difference is significant at $p < 0.05$.

and 3.00 nM (specific activity, 35.9 Ci/mmol). Specific binding was defined as the total binding minus that which occurred in the presence of 1 µM d-butaclamol. Scatchard graphs of data for binding of (^{3}H) spiroperidol were used for calculating the density of receptor sites (B_{max}) and the dissociation constants (K_d). In order to make each Scatchard plot the brain regions of 4 rats were pooled after homogenization. Student's t-test was used for statistical analysis. P-values less than 0.05 were considered significant. As shown in the table the Bmax's found in neostriatum, midbrain, and frontal cortex of VEE infected rats were significantly lower than those observed in controls. The decrease was more accentuated in frontal cortex (44%).

In the past few years we have accumulated some information regarding the neurochemical abnormalities of VEE virus infection[2-4,12]. As a result of these studies, it looks probable that the dysfunctions are due to degeneration of neurons, especially those located within the substantia nigra and basal ganglia which utilize dopamine, GABA and acetylcholine as their neurotransmitters. Because of the neuronal loss and cavitary necrosis observed in the brain of VEE infected rats[13], it was considered possible that the receptors for these neurotransmitters were to some extent damaged during the infection.

The reduction in specific receptor binding shown by this report appears then to be due to neuronal loss. It could also result from a decreased receptor affinity rather than from a fall in the density of receptors. However, the K_D's obtained from the VEE infected rats were not significantly different from the K_D's found in controls.

The significant reduction produced in the number of dopamine and/or serotonin receptor sites in different brain regions as a result of VEE viral infection might explain some of the neurological symptoms and behavioral responses (irritability, hyperthermia, tremor, ataxia, paralysis, and coma) observed in infected rats.

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Differential sensitivity to ethidium bromide of replicative DNA synthesis and bleomycin-induced unscheduled DNA synthesis in permeable mouse sarcoma cells[1]

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Summary. Replicative DNA synthesis in permeable mouse sarcoma cells was more sensitive to ethidium bromide (EtBr) than bleomycin-induced unscheduled DNA synthesis (UDS). A similar difference in sensitivity to EtBr was observed between DNA polymerases α and β. The difference in sensitivity to EtBr of replicative DNA synthesis and UDS in the present system seems to reflect mainly the sensitivity difference between DNA polymerases α and β.

Ethidium bomide (EtBr) which intercalates in double stranded regions of DNA and RNA has been shown to affect the activities of various enzymes involved in DNA and RNA metabolism[2-11]. Of eukaryotic DNA polymerases, DNA polymerase γ (mitochondrial DNA polymerase) has been shown to be highly sensitive to the drug[2,5,7,9]. There have been only a few reports on the effects of EtBr on eukaryotic replicative DNA synthesis, unscheduled DNA synthesis (UDS) and activities of DNA polymerases α and β. Mattern and Painter[10] reported that EtBr gave a biphasic dose effect, stimulation at low concentrations and inhibition at higher concentrations, on replicative DNA synthesis in permeable Chinese hamster ovary cells. The present experiments were performed to see whether or not a biphasic effect of EtBr on replicative DNA synthesis and UDS in permeable mouse sarcoma cells exists, and whether or not there is a difference in sensitivity to EtBr be-