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Cerebral serotonin in viral encephalitis

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Summary. In order to evaluate central serotonergic function during viral encephalitis biochemical, behavioural and immunohistofluorescence studies were carried out. Mice were inoculated with the moderate virulent strain of venezuelan equine encephalomyelitis virus, Pixuna. Signs of encephalitis were observed in 50–60% of infected animals. Levels of serotonin and 5-hydroxyindolacetic acid, and the ratio of the indolamine and its metabolite in raphe and cortex did not change with respect to sham-inoculated mice. A differential decrease in turnover rate by pharmacological methods, such as pargyline, p-chlorophenylalanine and probenecid administration, was observed in raphe and cortex. The ratio serotonin turnover rate/steady state concentration of serotonin was only decreased in the raphe of sick animals. The response to 5-methoxy-N,N-dimethyltryptamine was greater in infected animals. The duration of immobility in the swim test was shorter in the infected group. A greater number of viral antigen particles was localized in raphe and periraphe areas than in cortex, brain stem or striatum. The results suggest a serotonin presynaptic deficit, a postsynaptic hyperreactivity of serotonin system, and a region-selective distribution of the virus.

Introduction

Viruses are capable to produce a wide variety of diseases and infect the nervous system in a number of distinct ways (Fields and Weiner, 1982). A virus can affect directly the epithelial cells in contact with the portal of entry, or can entry by a primary place and disseminate using blood stream, lymphatics, or peripheral nerves for spread (Sharpe and Fields, 1985; Tyler et al., 1986). Then, the virus localizes in specific tissue or areas of a determined tissue following its selective tropism. Examples of these viruses are poliomyelitis, hepatitis, varicella-zoster, equine encephalitis, reovirus (Fields and Green, 1982; Johnson, 1982; Kristensson, 1982; Sharpe and Fields, 1985; Walder and Bradish, 1979).

The basis of neurotropism and the pathogenesis of CNS dysfunctions in viral infections is unknown. It has been assumed that cells do not posses specific receptors for viruses, but that cell receptors have evolved as attachment sites for viruses (Johnson, 1982; Maratos-Flier et al., 1983). Today, the great advance

in the molecular basis of neurotropism has helped to understand the mechanisms for some viral systems (Lipkin et al., 1988).

There are a number of reports suggesting a cause of certain neurological and psychiatric disorders by infectious agents (Allen et al., 1987; Crow et al., 1979; Crow et al., 1983; Kaufman et al., 1983; Koehler and Guth, 1979; Meijer et al., 1988; Miyasaki et al., 1977; Rhodes et al., 1984; Torrey, 1988; von Economo, 1931). Researchers are searching for the "schizovirus" (Torrey et al., 1988), that is psychosis might be the result of the expression of a virus sequence which is integrated in the genome (Crow, 1984, 1987). Moreover, certain reports concerns the preference of various central nervous system (CNS) viral infections for localized brain areas (Damasio and Vanhoese, 1985; Delamonte, 1985; Delsedime et al., 1984; Fishman et al., 1985; Lipkin et al., 1988; Maurizi, 1984, 1985a, b; Simantov et al., 1976; Tyler, 1986).

Venezuelan equine encephalomyelitis (VEE) is caused by an arbovirus and is distributed from Southern United States to Brazil. The majority of cases in endemic areas of VEE in North America (Florida strain) are asymptomatic, because a great number of residents have neutralizing antibody titers and no history of illness (Tyler, 1984). Certain neurological sequelae in residents of venezuelan endemic regions of VEE virus (VEEV) has also been reported (León et al., 1975).

Guajira strain of VEEV exerts neurochemical changes in determined regions of mice and rats CNS (Bonilla et al., 1980, 1982, 1984, 1986; Levine et al., 1981). Moderate virulent or avirulent strains of VEEV also produce selective decreases in catecholamine turnover rates in mouse brain (Lima et al., 1983). Herpes simplex virus infections alter monoamine metabolism (Lycke et al., 1968, 1969, 1970, 1971). Mumps virus infection of human medulloblastoma cell line and rat pheochromocitoma alters voltage-gated sodium channel and produces, and increase in the synthesis and release of prostaglandins E_1 and E_2 (Ziegler et al., 1988). Alterations of sleep stages in mice infected with rabies virus was also reported (Tsiang et al., 1988).

The relation of serotonin (5HT) with mental states has been discussed and supported through the years (Soubrié, 1986; van Kammen, 1987). The purpose of these studies has been to explore central serotonergic function during a moderate encephalitis with mild damage of the tissue, and to determine a region-selective brain localization of the virus.

Materials and methods

Animals

Male NMRI/IVIC mice (20 ± 5 g), 24 days old were used. The animals were fed ad libitum, maintained in groups of 5 per cage or in individual cages for the behavioural studies, in a room under controlled lighting (12 h on/12 h off) and temperature. A background noise was provided by the air extractor. At the end of the experiment surviving animals were challenged with a lethal dose of the strain P-2023 of VEEV in order to verify the infection. Groups of animals were designated as control (C), infected with no signs (INS), and infected with signs (IS).

Virus

Pixuna strain of VEEV was inoculated intracranially (ic) in 0.03 ml of bovine serum albumin at a dose of 300 LD_{50} for biochemical and behavioural studies, and 1,000 LD_{50} for immunofluorescence test, determined in suckling mice. Control animals were inoculated with the vehicle (Walder and Bradish, 1979).

5HT turnover rate

5HT and 5HIAA were determined by reversed phase HPLC with electrochemical detector as previuosly described (Lima et al., 1987). Mice were sacrifecd by decapitation at 4, 7, 15, 21, 30, and 60 days after inoculation and tissue was dissected in frontal cortex and raphe area (Lima et al., 1987). Three pharmacological methods were used for turnover determination, the administration of the monoamine oxidase inhibitor pargyline (Sigma), the tryptophan hydroxylase inhibitor *para*-chlorophenylalanine (PCPA, Sigma), or the acid transport inhibitor probenecid (Palenzona). 5HT/5HIAA ratio was calculated. The turnover rate/$[5HT]_0$ ratio is reported as an index of the density of serotonergic innervation (Meek and Lofstrandh, 1976).

Behavioural measurements

The open field test was carried out as previously described (Gersson and Baldessarini, 1982; Lima et al., 1988). The serotonergic syndrome was evaluated (Jacobs, 1976) after the ip administration of 5-methoxy-N,N-dimethyltryptamine (DMT), or 5-hydroxytryptophan (5HTP) (Graham-Smith, 1971; Lima et al., 1988). The swim test was done according to Porsolt et al. (1977).

Immunofluorescence test

Sections (10 μm) of brain stem, striatum, frontal cortex and raphe area (Aghajanian et al., 1973; Glowinski and Iversen, 1966) 24, 48, and 72 h post-inoculation were prepared on a cryostat-microtome. Indirect fluorescent antibody test was performed by using mouse ascitic fluid (MAF) against VEEV (strain P-2023) as primary antibody, normal MAF and MAF against Dengue virus (Monath et al., 1981). Controls were noninfected mice and Vero cells. Stained slides were examined with a Zeiss epifluorescence microscope.

Results

The 50–60% of mice developed signs of lethargic encephalitis between 6 and 8 days after inoculation. Sick animals died and some infected appeared apparently unaffected.

5HT turnover rate

Values of 5HT and 5HIAA remained under steady state levels in control and infected group of animals at all days post-inoculation. The 5HT/5HIAA ratio was not significantly different among the groups of mice. The administration of pargyline produced a time-dependent increase in 5HT content of raphe area and cortex of C animals (Fig. 1). This increase is not statistically significant in the raphe area of INS and IS groups (Fig. 1 A, B). In the cortex the accumulation of 5HT is statistically significant in all groups of mice by 15 minutes after

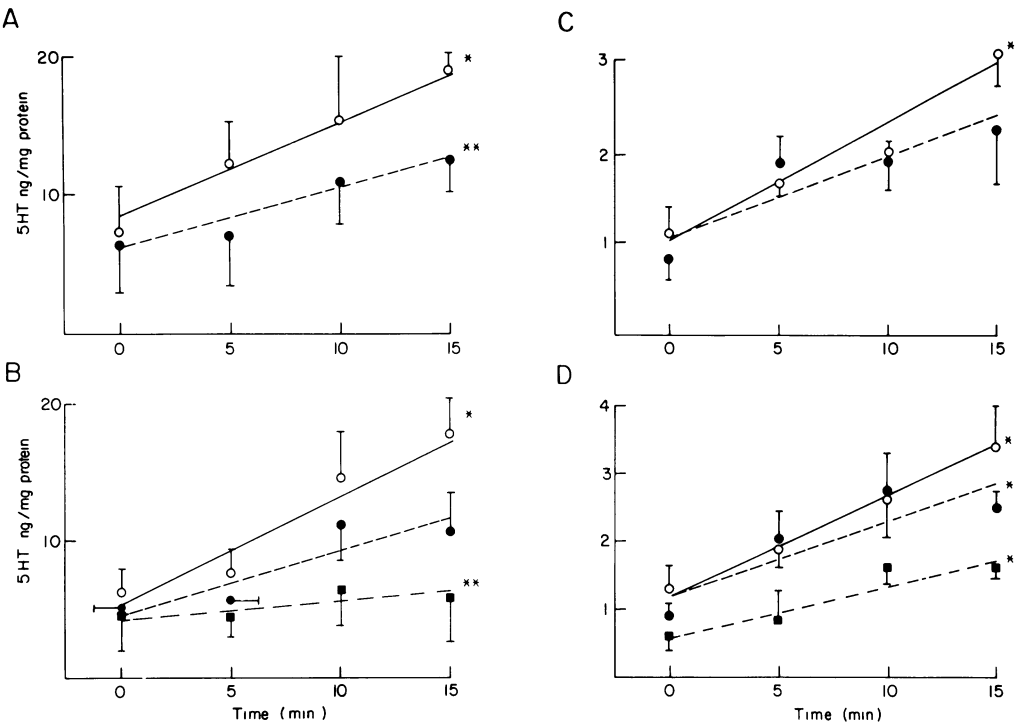

Fig. 1. Time-dependent effect of pargyline (100 mg/kg ip) on 5HT concentration in raphe and cortex. C, control (○); INS, infected with no signs (●); IS, infected with signs (■). Each value represents mean ± SE, n = 4–9. Turnover rate is express in ng·mg prot^{-1}·h^{-1}: **A** raphe 4 days post-inoculation, 44.24 in C, 25.72 in INS; **B** raphe 7 days post-inoculation, 47.53 in C, 26.84 in INS, 5.16 in IS; **C** cortex 4 days post-inoculation, 7.08 in C, 4.88 in INS; **D** cortex 7 days post-inoculation, 9.08 in C, 6.36 in INS, 4.76 in IS. ANOVA and linear regression analysis were carried out (Sokal, 1979). * $P<0.05$ with respect to corresponding 0 time. ** $P<0.05$ with respect to control at 15 min

Table 1. Turnover rate/$[5HT]_0$ ratio in raphe and cortex

Days post inoculation	Brain area	Groups of animals		
		C	INS	IS
4	Raphe	6.32 ± 2.43	4.93 ± 2.56	—
7		7.41 ± 1.09	6.02 ± 2.67	1.28 ± 0.68*
4	Cortex	6.15 ± 0.92	6.60 ± 2.06	—
7		8.37 ± 2.06	7.00 ± 1.24	7.80 ± 2.94

Each value represents mean ± SE. *C* control; *INS* infected with no signs; *IS* infected with signs; n = 4–8. Probability was calculated by ANOVA (Sokal, 1979).
* $P<0.001$ with respect C at 7 days

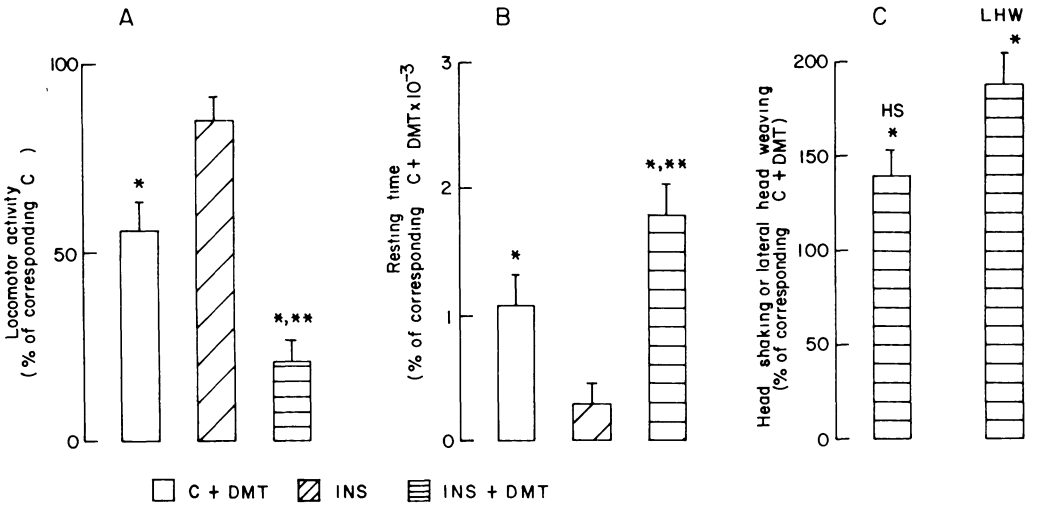

Fig. 2. A Locomotor activity. **B** Resting time. **C** Head shaking (HS) and lateral head weaving (LHW) in control mice, C and infected with no signs, INS after the administration of DMT (2.5 mg/kg ip). Animals were observed for 11 min. Each value represents mean ± SE, n = 20. ANOVA was carried out (Sokal, 1979). * $P < 0.001$ with respect to C in A and B or with respect to C + DMT in D. ** $P < 0.001$ with respect to C + DMT

pargyline, but the slope of increase differed from C group (Fig. 1C, D). The administration of probenecid or PCPA also show a decrease in the turnover rate of 5HT (Lima et al., 1987).

Density of 5HT innervation

The turnover rate of $5HT/[5HT]_0$ ratio, as an index of the density of 5HT innervation (Meek and Lofstrandh, 1976), did not change among all groups except in the raphe of sick animals (Table 1).

Table 2. Swim test in control and infected mice 4 days after inoculation

Group of animals	Periods of observation (sec)		
	1st	2nd	Total
C	42.42 ± 12.12	103.03 ± 9.09*	151.51 ± 15.15*
INS	15.15 ± 6.06	42.00 ± 10.60**	69.60 ± 11.21**

Each value represents mean ± SE. n = 10–20. *C* control; *INS* infected with no signs. Periods of observation: 1st, from 1–4 min, and 2nd, from 5–7 min after placing the mouse in the water. Probability was calculated by Student's t-test (Sokal, 1979).

* $P < 0.01$ with respect to C, 1st.

** $P < 0.001$ with respect to C, 2nd or total

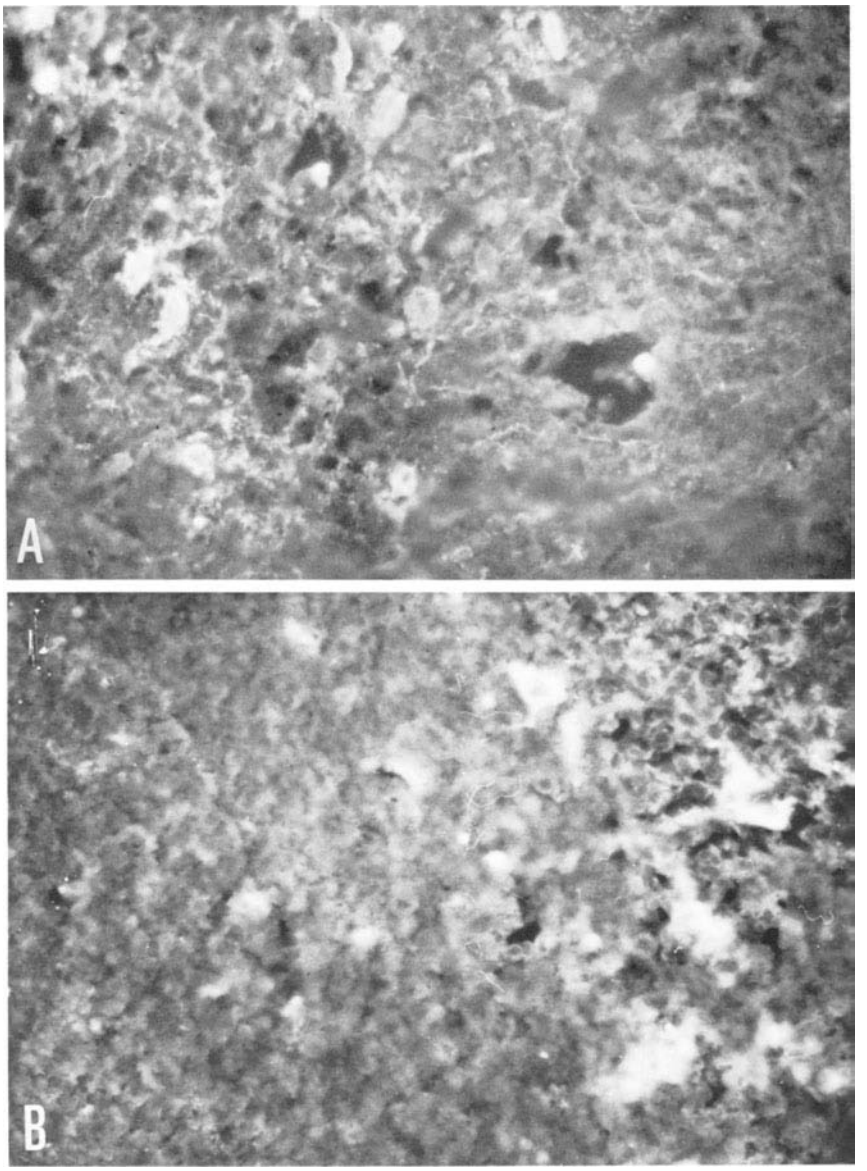

Fig. 3. Frozen sections of raphe (**A**) and cortex (**B**) from mouse brain showing specific VEEV immunofluorescence (25 ×)

Serotonergic syndrome

Locomotor activity of vehicle and virus-inoculated mice did not present any statistical significant difference at 4, 5, 6, 7, 15, 21, and 30 days post-inoculation. However, infected mice presented an exagerated response to the administration of DMT as is indicated by parameters such as locomotor activity, resting time, head shaking and lateral head weaving (Fig. 2). This effect is not observed by the administration of 5HTP to virus-inoculated mice (Lima et al., 1988).

Swim test

During the first 3 min of observation control animals presented vigorous activity and then a progressive increase in immobility. Infected mice were continuously moving up to 7 min of observation (Table 2).

Immunofluorescence of viral antigen

24 h after inoculation there was a generalized distribution of viral antigen in brain tissue mainly localized in microglia. 48–72 h post-inoculation neurons presented a concentration of viral antigen with a heterogeneous distribution, low in cortex, striatum and brain stem, around 7 cells per field (25 ×). In dorsal raphe area were 15 cells per field, medial raphe area, 8 and regions periraphe area, 8 cells per field (Fig. 3).

Discussion

Indirect evidences of the effect of viral infection on CNS metabolism have been reported concerning protein synthesis, catecholamines, acetilcholine, 5HT, dopamine receptors, among others (Bonilla et al., 1980, 1982, 1984, 1986; Tsiang et al., 1988; Ziegler et al., 1988). In addition, the specific tropism of certain viruses has been demonstrated in some models (Fields, 1982; Lipkin et al., 1988). Moreover, a selective distribution of viral antigen has been shown for coronavirus; eastern equine encephalitis and retrovirus (Fishman et al., 1985; Monath et al., 1981; Tyler, 1986).

The advantage of using a moderate virulent strain of VEEV is that there are infected animals which will not present signs of encephalitis, with mild histological changes in brain tissue (Lima et al., 1983, 1987). Moreover, the fact that a decrease in 5HT turnover rate is observed prior to the appearance of the first signs of the illness indicate that biochemical changes precede the development of clinical manifestations.

The selectivity of monoamine is another subject. Even if all catecholamines decrease (Lima et al., 1983) and 5HT turnover rate too, there is a differential effect in terms of time-curse, region and monoamine (Lima et al., 1983, 1987). Some of the biochemical changes reported to occur during encephalitis by VEEV are dependant on the region of the brain (Bonilla et al., 1984; Levine et al., 1981; Lima et al., 1983). Other viruses have been reported to have a preference for certain brain areas such as limbic system (Damasio and Vanh, 1985), basal ganglia (Fishman et al., 1985), locus coeruleus and raphe area (Maurizi, 1985a, b).

The decrease in 5HT turnover is mainly observed in the raphe area (Fig. 1 A and B) and at 4 and 7 days post-inoculation. In the cortex the administration of pargyline produces an accumulation of 5HT in control and inoculated mice, but the slope of the increase is different and the turnover rate differs (Fig. 1 C and D). At the fourth day post-inoculation the turnover rate of 5HT decreased in 43% in raphe and 31% in cortex. By the 7th day the decreases in turnover

are of 43 and 89% the raphe area, and of 30 and 47% in the cortex for INS and IS groups, respectively. Thus, raphe area is more sensitive to the action of the virus than cortex. Moreover, the decrease in 5HT turnover in raphe and cortex in infected animals without signs of encephalomyelitis with respect to sick animals are evidence that the measurement of biochemical markers could be indicative of the severity of the disease.

The observed defficiencies could be the product of destruction of the cells, however the index given by the ratio turnover rate/$[5HT]_0$ as a parameter of density of 5HT innervation (Meek and Lofstrandh, 1976) only presented statistical significant decrease in the raphe area of IS group of mice (Table 1). This is another indication of the relative higher vulnerability of raphe area than cortex, despite the fact that ic inoculation, facing the cerebral cortex at first, was carried out. Moreover, the biochemical observations in the raphe and cortex of INS animals or in the cortex of IS group do not correspond to lost of cells.

The hyperreactivity of infected animals (INS) to the 5HT agonist, DMT is indicative of a supersensitivity of 5HT receptors (Corne et al., 1963; Soubrié, 1986) (Fig. 2). On the other hand, the administration of 5HTP, 5HT precursor, which might need an intact terminal to exert its effect, does not produce an exageration of the serotonergic syndrome (Lima et al., 1988). The results suggest a decrease in the presynaptic function and a hyperrresponse at the post-synaptic level.

The swim test is modified by tryptophan administration, which increases brain 5HT and produces an increase in immobility (Gibson et al., 1982). Infected animals (INS) display vigourous movements in the water and a decrease of immobility with respect to control group (Table 2), just the opposite than the effect of tryptophan.

Preliminary experiments searching for the distribution of viral antigen in certain brain areas have been carried out by immunofluorescence test. Of the four regions analysed the raphe and periraphe area presented the highest concentration of viral immunoreactivity in neurons (Fig. 3). Once again the preference of raphe manifestations are shown by the localization of the virus. The mechanisms by which this virus migrate to deep areas of the brain are unknown. Also the stay for longer periods of time in specific areas of the brain needs to be investigated.

Viral infections deal to a complex response in which several mechanisms are involved. The preference of virus for certain areas of the brain and the differential biochemical response to the infection suggest a selectivity that can have biological significance related to behaviour and pathology. In addition, the changes in neurotransmitters produced by certain viruses could be useful in pronostic and diagnostic of sequeleae post-encephalitis.

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