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THE EFFECT OF DEHYDROEPIANDROSTERONE (DHEA) ON SURVIVAL OF MICE INOCULATED WITH WEST NILE VIRUS AND EXPOSED TO COLD STRESS.

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SUMMARY

The effect of Dehydroepiandrosterone (DHEA) in protection from stress-enhanced viral encephalitis was studied in mice exposed to cold stress and inoculated with West-Nile virus (WNV). Mice were checked for mortality, blood and brain virus levels. Exposure of WNV inoculated mice to cold water (1±0.5°C) stress (5 minutes/day for 8 days) resulted in a mortality rate of 96% as compared to 45% in control mice. Administration of DHEA (serial injections of 10-20 mg./kg. with or without a loading dose of 1 gr./kg.) resulted in a reduction of the mortality rate to 40%-50%. Virus levels in the blood were lower in two logs in the DHEA treated mice, brain virus levels (7 days following inoculation) were significantly lower in the DHEA treated group. DHEA was again found to be effective arresting the stress-induced involution of lymphoid organs.

The present study provides direct evidence of the protecting effects of DHEA as "anti-stress" agent. Its ability to prevent mortality (against WNV or WN-25) and lymphoid organs involution caused by stress induced immunosuppression supports the notion that its activity is based on the counteractoin of glucocoticoids.

INTRODUCTION

Dehydroepiandrosterone (DHEA) is one of the major adrenal cortical steroid hormones in mammals and in humans (Migeon et al. 1957, Sonka et al. 1964, Windholz 1976).

DHEA is quantitatively the major secretory product of the human adrenal gland and the levels of this hormone begin to decline after the second decade of life reaching 5% of the original level in the elderly (Barret-Connor et al. 1986, Wang et al. 1971). DHEA's function as a weak androgen suggests that its major biological role is that of a

facultative precursor for other steroids and may have an alternative biological activity in other systems (Rosenfeld et al. 1971).

Recently, DHEA was suggested to have an immunomodulating activity. It was found that DHEA can prevent dexamethasone-induced thymic involution (May et al. 1990), increase IL-2 production (Daynes et al. 1990), and prevent the development of Systemic Lupus Erythematosus in mice (Lucas et al. 1985).
In vivo studies have suggested that DHEA protect mice from various lethal viral infections (Loria et al. 1988, Ben-Nathan et al. 1991). However, DHEA did not show any antiviral effect in-vitro. The mechanism of protection is unclear but, it was suggested that DHEA protects mice by counteracting the immunosuppressive effects of glucocorticoids (GC). GC are major mediators in the reaction to stress and in stress-induced immunosuppression (Riley, 1981. Khanshari et al. 1990, Dantzer and Kelley 1989). Administration of GC during viral infection lead to higher viral titers and increased morbidity and mortality (Riley 1981, Grossman 1985). The effect is similar to that of stress on viral infections (Rasmussen et al. 1957, Ben-Nathan and Feuerstein 1990).
In general the administration of glucocorticoids during viral infection caused immunosuppression (Ben-Nathan et al. 1989, 1990, Gianelly and Terner 1968, Riley 1981) leading to higher viral titers and increased symptomatology and mortality (Riley 1981, Grossman 1985).

Environmental or physical stress is known to affect the immune system. For example, dexamethasone or cortisol injections (Parillo and Fauci 1979) cold or isolation stress (Ben-Nathan and Feuerstein 1990, Ben-Nathan et al. 1989, Ben-Nathan et al. 1991) can cause involution of lymphoid organs such as thymus, spleen and lymph nodes through activation of the pituitary adrenocortical axis (Sapse 1984, Friedman et al. 1970), Interferon production is suppressed in stress situations (Jensen 1973), and T-helper lymphocyte levels are reduced under various types of stress (Kiecolt-Glaser et al. 1984, Sapse 1984). In fact, stress paradigms such as avoidance behavior has already been shown to exacerbate several infectious agents, including Herpes Simplex (Rasmussen et al. 1957), Coxakie B_1 (Johnson et al. 1963) Vesicular Stomatitis Virus, (Jensen and Rasmussen 1963), and Encephalitis Viruses (Ben-Nathan et al. 1989, 1991). It is known that stress conditions affect the immune system and cause immunosuppression (Friedman and

Glasgow, 1966. Johnson et al. 1963) through involution of lymphoid organs (Ben-Nathan et al. 1989, Parillo and Fauci 1979) possibly mediated by increased activity of the pituitary adreno cortical axis (Friedman et al. 1970, Sapse 1984). It appears that stress situations act by enhancing virus replication in lymphocytes as shown in the spleen of stressed mice. Since lymphocytes can pass through the blood brain barrier, the larger fraction of infected lymphocytes in stressed mice could contribute to higher virus titer also in the brain. This possibility however, awaits further clarification by more direct evidence on the mode of immune cell penetration into the CNS. In contrast, DHEA was shown to prevent mortality in infected mice with viruses such as Coxackie virus B_4 Herpes Simplex Type 2 or Encephalitis viruses (Loria et al. 1988, Ben-Nathan et al. 1991). It was suggested that DHEA may counteract the immunosuppresive effect of glucocorticoids (Loria et al. 1988, Riley 1981) which are elevated during viral infections (Ben-Nathan and Feuerstein 1990, Blalock 1987, Yirrel et al. 1987).

West Nile Virus (WNV) is a member of the flavivirus genus, of the family Flaviviridae. As a flavivirus it contains a non-segmented single stranded 42S RNA and 3 virion polypeptides, one of which is a glycoprotein (Westaway et al. 1985). WNV is widely distributed throughout Asia, Africa and parts of Europe (Monath 1986), WNV is a neurotropic arbovirus (Weiner at al. 1970) and is capable of endemic spread (Goldblum et al. 1954, Hayes et al. 1982). Wild birds are the primary host but high antibody rates in a variety of animals including man, indicate a broad infection spectrum; in man the morbidity rate is low and severe cases of encephalitis occur only occasionally, but sub-clinical infection is common (Chamberlin 1980). In parts of Africa up to 70% of the human population may possess antibodies (Chamberlin 1980).

A variant of West Nile Virus, designated WN-25 has been isolated from a WNV-persistent infection in mosquito cell cultures (Halevy et al. 1988). The variant WN-25 virus showed no differences from WNV in serology tests (HI, NT and RIA), buoyant density, surface charge and RNA fingerprints. Both strains grew equally well in BHK and Vero cell cultures.

The ratios of plaque forming units to HA, antigenic mass (RIA), and LD_{50} by intracerebral (I.C.) route in suckling and adult mice were similar in both strains. The lethality of both strains in suckling mice by the I.P. route was similar. In adult mice, WNV was as lethal by I.P. as by I.C. inoculation, whereas WN-25 was completely non-lethal when injected I.P. No infectious virus could be found in the brains of the WN-25 injected mice although all became immune (Halvey et al. 1988).

In previous studies (Ben-Nathan et al. 1989) we have shown significant differences in mortality rates from WN-25 infection in stressed versus non-stressed mice.

The aims of the following study are:

1. to explore whether Dehydroepiandrosterone (DHEA) can prevent the negative effects of cold stress on the course of West Nile virus encephalitis.
2. to examine if DHEA can prevent the involution of lymphoid organs caused by cold stress.

MATERIALS AND METHODS

Viruses

West Nile Virus: The original strain of virus was isolated from a human case of WNV infection (Goldblum et al. 1954). The virus stock was prepared and assayed in Vero cells in our laboratory. The virus stock used for the experiments contained $3x10^8$ plaque forming units (PFU/ml.). The intracerebral titer (LD_{50}) was $1.3x10^7$/ml. and $6.9x10^6$ mouse I.P. LD_{50}/ml.

WN-25: Aedes aegypti cultures (kindly provided by Dr. Peleg) were infected with an input of about one PFU per cell of WNV from mouse brain suspension. The infected culture was subcultured once or twice a week and the virus progeny was harvested. At passage 25 the virus was plaque purified on Vero cells (three times) regrown in C6/36 (Aedes albopicus cell line) and later on Vero cells (Halevy et al. 1988).

Mice

Charles River outbred ICR female mice (CD1) were obtained at the age of 21 days (10-12 gr. body wt.) and kept in our vivarium until the age of 27-30 days. In all studies mice of the same age and batch were used.

Virus inoculation: Each mouse was inoculated with 0.2 ml. (I.P.) of West Nile virus containing 10-100 PFU or with WN-25 200,000 PFU/mouse.

Cold stress: Mice were placed for 5 min./day in cold water (1±0.5°C). The mice could stand in water, which was 3 cm. deep. WNV or WN-25 virus was inoculated and immediately after, the mice were exposed to cold stress. Stress continued every day until 8 days post inoculation. For mortality rate, mice were observed for 21 days and for brain virus level measurment, mice were sacrified 7 and 8 days after inoculation.

Activity of DHEA (Sigma) in vivo: DHEA was suspended in RSSP for I.P. injection or in dimethyl-sulfoxide (DMSO) or paraffin oil for S.C. injection. The mice were injected I.P. with low doses of the drug (5-20 mg./kg.) at days 2, 1, 0 before and on days 2, 4, 6 and 8 after virus inoculation. Mice treated with high doses of DHEA (0.2-1 gm./kg.), were injected S.C. one day before and on day 3 and 6 after virus inoculation. Whereas 20 mg./kg. were injected I.P. at days 1, 0 before and on days 2, 4, 6 and 8 after virus inoculation.

Dexamethasone (Sigma D-1756) was diluted in saline and was injected I.M. 2 mg./kg. two hours before and one day after virus inoculation.

Tissue Culture

Vero cells: the Vero cell line was derived from kidneys of a normal African Green Monkey. The cells are grown in Dulbeco's Modified Eagle Medium (DMEM) containing 10% fetal calf serum (FCS).

BHK cells: A baby hamster kidney (BHK) cell line is grown in Eagle's F-12 medium supplemented with 10% tryptose broth and 10% calf serum.

Corticosterone: plasma corticosterone was assayed by radioimmunoassay (ICN Biomedicals, Inc. CA, U.S.A)

Organs' Weight

Mice were individually weighed at 7 days after inoculation and exposure to cold stress. The mice were sacrified and the thymus and spleen immediately removed and weighed aseptically.

Isolation of WNV from the spleen and brain of infected mice.

The spleen and brains of each group were individually rinsed in cold PBS (1 ml. per spleen and 2 ml. per each brain) containing 2% FCS and Penicillin (1,000µg./ml).

Each brain or spleen was rinsed in cold PBS and sonicated. The virus suspension was centrifuged at 3,000 rpm for 10 minutes. The supernatant was aliquoted into plastic tubes and stored at -70°C until further processes. Virus levels in blood, brain and spleen are determined by titration of virus in Vero cells or in BHK cell line.

Titration of Virus in tissue cultures.

For demonstration of WNV plaques in Vero cells the original plaque technique of Dulbeco and Vogt was used. A dilution of virus is added to Vero cell monolayers in petri dishes and incubated at 37°C for 1 hour to permit viral adsorbtion. The monolayer is overlaid with MEMx2 and Tragacanth containing 2% FCS and 2.4% $NaHCO_3$. Cultures were incubated (37°C, 5% CO_2) for 72 h. Plaques are counted after staining the monolayer with neutral red (0.05%). The same procedure was followed for BHK cells. All plaques were counted by an experienced investigator.

Data Analysis

All data in text and figures are mean values ± SEM for the indicated number of mice. Data were analyzed by ANOVA and the Student-Newman-Keul test for a-posteriori multiple comparisons or the Kruskal-Wallis followed by Mann Whitney U-test were appropriate.

RESULTS

This project was aimed to evaluate the effects of DHEA as an anti-stress agent on the course of West Nile virus encephalitis in mice. Furthermore, DHEA's capability to prevent the adversive effects of stress and of viral infection at several stages was estimated.

<u>The effect of cold stress on body temperature and corticosterone level.</u>
The stress paradigm used in this study is cold exposure. Mice were exposed to cold water (1±0.5°C) for 5 minutes. This treatment caused a decrease in body temperature to 14°C, which gradually returned to nearly normal after two hours (Fig. 1). Corticosterone levels an accepted parameter to assess stress effects showed a marked increase reaching a maximal plasma level of 480 ng./ml. Differing from the body temperature situation, the corticosterone levels remain higher than normal even after 24 hours (218 VS 95 ng./ml. Fig 1).

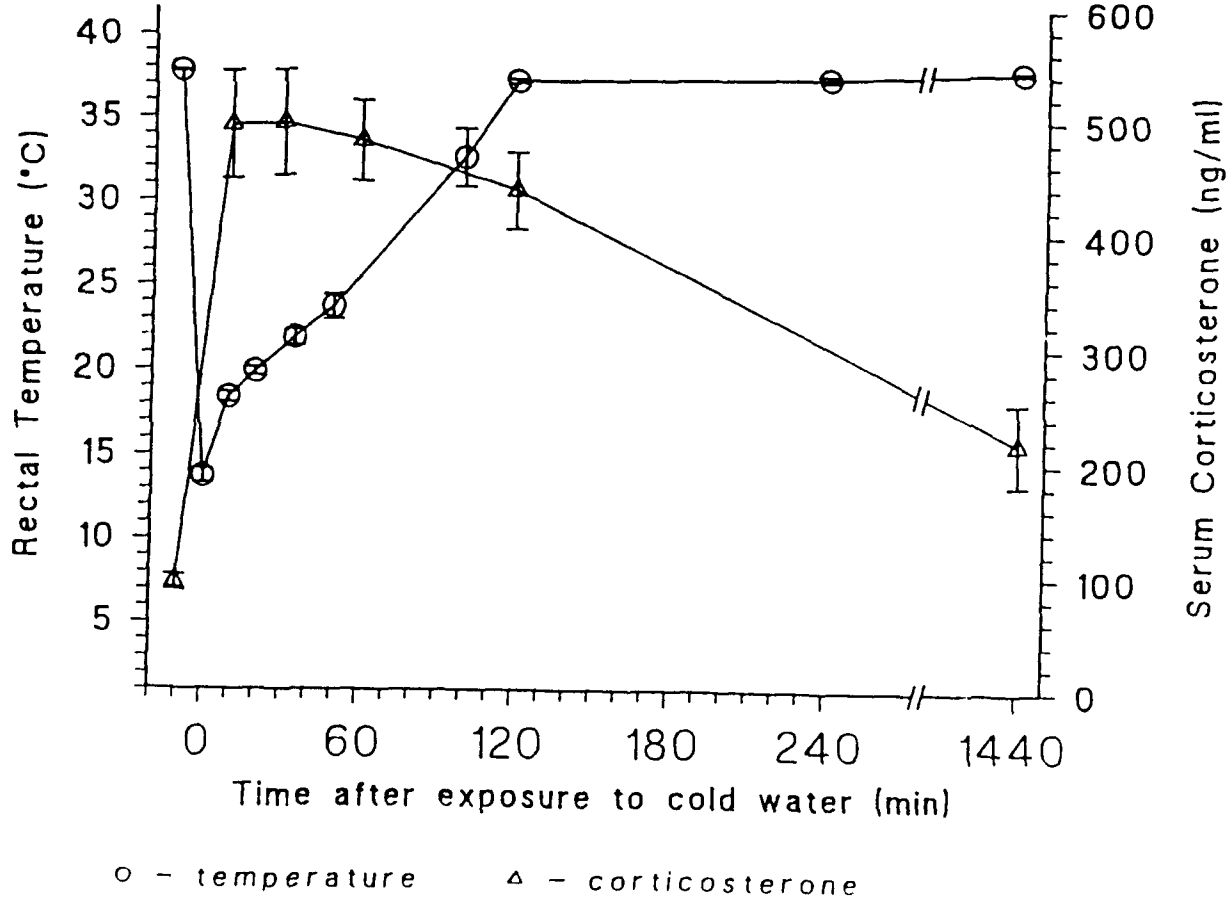

Fig. 1 - Effect of cold stress on body temperature (N=9) and plasma corticosterone level (N=6).

The effect of DHEA on lymphoid organs weight in cold exposed mice.

Stress is known to induce involution of lymphoid organs such as thymus, spleen and lymph nodes (Riley 1981). This involution serves as a common parameter to assess the immunosuppressive effect of stress.

The purpose of these experiments was to determine if DHEA can prevent this stress induced involution. Body and organs' weight was measured 7 days after virus inoculation and/or exposure to cold stress in DHEA treated and non treated mice. Table 1 presents the thymus and spleen and and body weights of mice inoculated with WNV (100 PFU/mouse) and/or exposed to cold stress. As shown, the weight of the spleen and thymus was significantly reduced in cold stress exposed mice. WNV inoculation of normal mice had no significant effect on spleen or body weight, but significantly reduced thymus weight. Cold stress alone, as was shown previously (Ben-Nathan and Feuerstein 1990), reduced organs' weights. The combined effect of cold stress with WNV tended to further reduce lymphoid organs weight. These reduction of lymphoid organs was antagonized by DHEA treatment (20 mg./kg.) and weight reduction was arrested at near normal level. Furthermore, increase in spleen and thymus weights was observed in all DHEA treated groups as compared to control groups. Fig 2 a, b shows the lymphatic organs weight of mice inoculated with attenuated WNV (WN-25) and exposed to different stress paradigms: cold (physical), isolation (social) or dexamethasone (pharmacological). The data clearly shows significant reduction of lymphoid organs caused by stress. DHEA treatment was found to be effective in all three paradigms of stress in preventing the involution of lymphoid organs. WN-25 injection had a weaker effect but, was synergetic to stress in reduction of spleen and thymus weights.

TABLE 1

Anti-stress effect of DHEA on spleen, thymus and body weight of mice 7 days after WNV inoculation and exposure to cold stress.

Treatment Group	Body weight (gm.)	Spleen weight (mg.)	Thymus weight (mg.)	N
Control	21.19±0.42	113.10±5.40	78.64±2.80	(12)
Control+DHEA+	20.85±0.35	145.00±6.40*	81.70±3.10	(12)
Cold	19.77±0.40	91.20±5.20	65.40±2.40	(12)
Cold+DHEA	20.60±0.50	182.80±12.4***	88.30±7.00*	(12)
Control+WNV	18.65±1.00	90.50±4.99	50.26±3.78	(10)
Control+WNV+DHEA	20.90±0.70	134.50±11.4*	69.00±3.94*	(10)
Cold+WNV	15.85±0.35	87.60±4.00	37.30±3.25	(14)
Cold+WNV+DHEA	19.09±0.48	150.52±10.8**	63.70±5.40**	(10)

* $p<0.05$ ** $p<0.01$ *** $p<0.001$ as compared to non treated group.

DHEA: 20 mg./Kg. I.P. on days -1, 0 before and on days 2, 4, 6 and 8 after virus inoculation.

Cold stress was introduced on the day of virus inoculation.

WNV: 100 PFU/mouse.

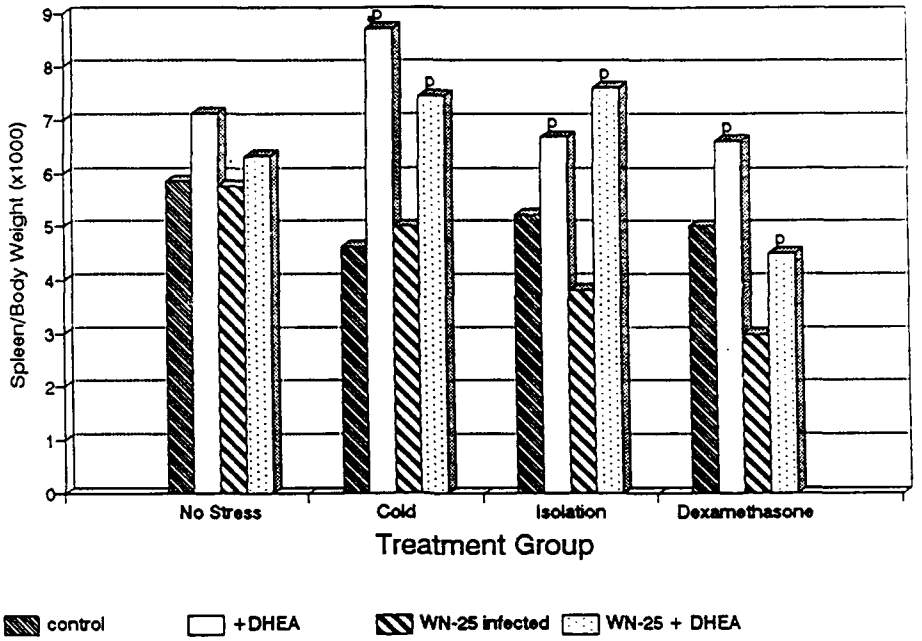

Fig. 2a- Effect of DHEA on splenic involution of mice 7 days after virus inoculation and exposure to various stress paradigms.
DHEA: 10 mg./kg. I.P. on days 1, 0 before and on days 2, 4 and 6 after virus inoculation and exposure to stress.
N=8, $p<0.05$ as compared to non treated group.

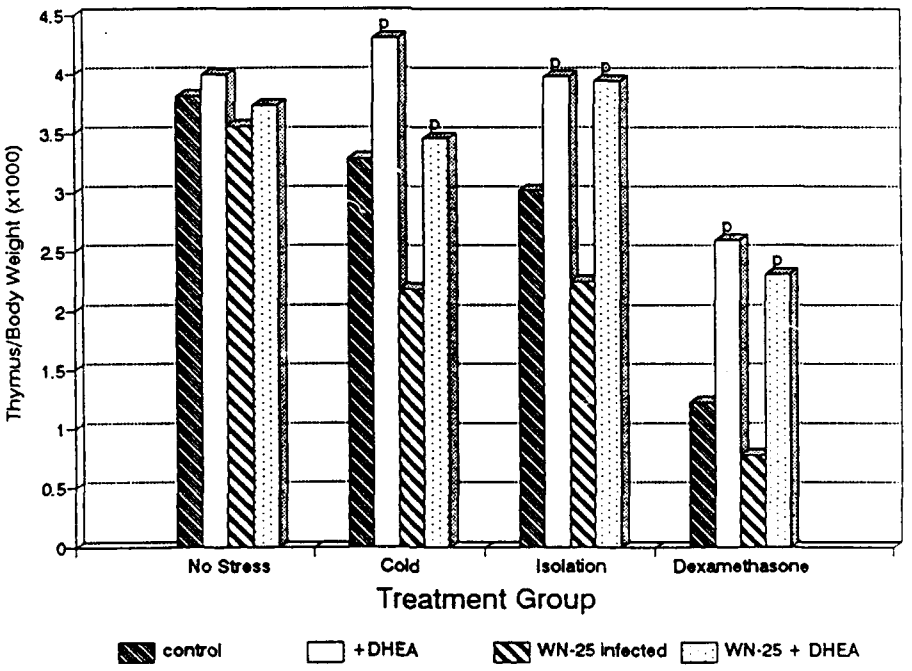

Fig. 2b- Effect of DHEA on thymic involution of mice 7 days after virus inoculation and exposure to various stress paradigms.
N=8, $p<0.05$ as compared to non treated group.

The effect of DHEA on mortality of mice inoculated with WNV or WN-25 and exposure to cold stress

DHEA was suggested to have an immunomodulating activity and may be protecting mice by counteracting the immunosuppressive effects of glucocorticoids. Therefore, the purpose of these experiments was to determine the effect of DHEA injection (5-20 mg./kg.) on the protection of mice from stress induced immunosuppression.

Each mouse was injected I.P. with DHEA on days 2, 1, 0 before and on days 2, 4, 6, 8 after virus inoculation (100 PFU/mouse). The results (table 2) show that DHEA protected mice against WNV infection. The percent of surviving mice following WNV infection was 20%-30% and 40% in DHEA (5, 10, 20 mg./kg.) treated mice respectively as compared to non survival of control mice. In stress groups DHEA administration reduced mortality rates from 100% to 80%, 60% and 40% in 5, 10, or 20 mg./kg. respectively. DHEA injection not only reduced death rate but also postponed the onset of disease and death by 2-3 days. Fig. 3 presented the effect of stress on mortality of mice inoculated with WN-25. Mice were injected with DHEA 10 mg./kg. I.P. on days -1, 0 before and on days 2, 4, 6 and 8 after virus inoculation. Mice were exposed to three different stress paradigms: cold (physical), isolation (social) or dexamethasone (pharmacological). The stress paradigms induced mortality of 67%, 75% and 67% respectively. DHEA administration reduced mortality rates to 22%, 32% and 11% respectively (Fig. 3). In non-stressed mice inoculated I.P. no mortality was seen with or without DHEA while I.C. inoculation caused 100% mortality (control I.C.). DHEA treatment prolonged the time of death to 9-12 days in stress exposed mice as compared to 7-10 days in stressed mice.

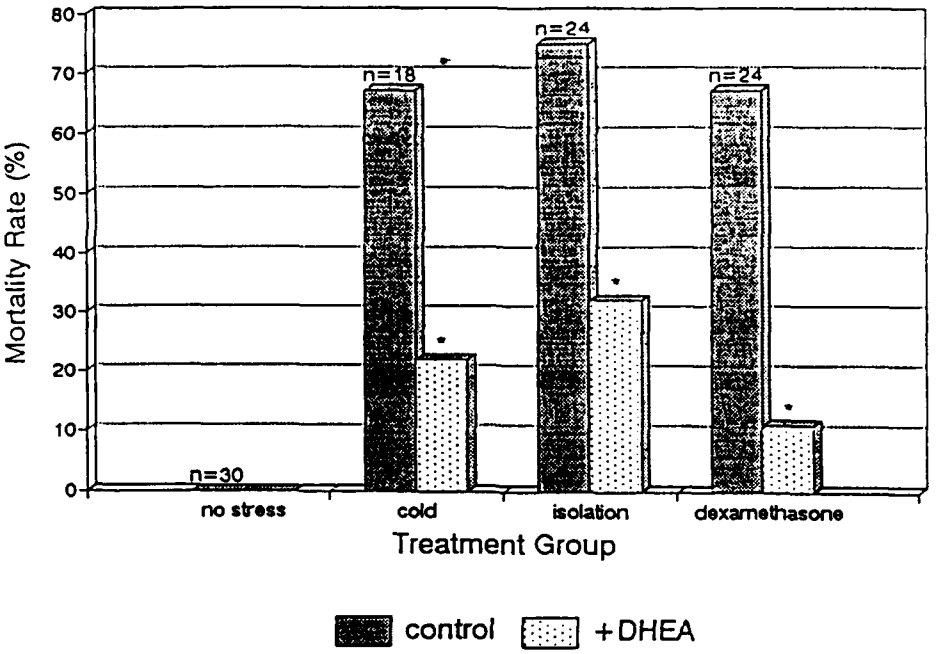

Fig. 3 - The effect of DHEA on mortality of mice inoculated with WN-25 virus ($2x10^5$ PFU/mouse) and exposure to various stress paradigms. DHEA: 10 mg./kg., I.P. on days 1, 0, before and on days 2, 4, 6 and 8 after inoculation and exposure. P<0.01 comapared to non treated group.

TABLE 2

The effect of DHEA on mortality of mice inoculated with WNV and exposed to cold stress.

Treatment Group	DHEA injected mg./kg.	Days after inoculation 7	8	9	10	11	12	13	14	D/T	% of dead
WNV	-	3	5	2						10/10	100
WNV+Cold	-	4	3	3						10/10	100
WNV	5	1	2	4	1					8/10	80
WNV	10	1	2	3	1					7/10	70
WNV	20		1	2	3					6/10	60
WNV+Cold	5		2	3	3					8/10	80
WNV+Cold	10		2	2	2					6/10	60
WNV+Cold	20			1	1	2				4/10	40*

* p<0.05 as compared to control group.

DHEA in RSSP I.P. on days -2, -1, 0, 2, 4, 6, 8 WNV 100 PFU/mouse.

Cold stress was introduced on the day of virus inoculation.

The effect of high dose injection of DHEA on survival of mice inoculated with WNV and exposed to cold stress

These experiments were done in order to determine the protective effect of high doses of DHEA 20, 200 or 1000 mg./kg. Moreover, we tested if there is a correlation between the dose of DHEA injection and the mortality rate. DHEA at a dose of 20 mg. was injected I.P. on days 1, 0 before and on days 2, 4, 6 and 8 after virus inoculation. The higher levels of 200 or 1000 mg./kg. were injected S.C. on day one before and on day 3 and 6 after virus inoculation (10 or 100 PFU/mouse). Table 3 presented the results of 2 experiments. As shown DHEA injection reduced mortality (10 PFU) from 50%-63% to 25%-38% in 20 mg., to 0 in 200 mg. and to 0%-13% in 1000 mg./kg. In those mice which were exposed to cold stress DHEA reduced mortality from 80%-88% to 40%-50%, 20%-38% and 25%-30% respectively. In mice inoculated with 100 PFU administration of DHEA reduced mortality from 90%-100% to 50%-60%, 20%-40% and 30%-50% in 20, 200 or 1000 mg./kg. respectively. In those mice which were exposed to cold stress, DHEA injection reduced mortality from 100% to 50%-60%, 40%-30% and 40% in 20, 200 or 1000 mg./kg. respectively.

TABLE 3

The protection by DHEA of mice inoculated with WNV and exposed to cold stress

Treatment Group	DHEA injected mg./kg.	EXPERIMENT 1				EXPERIMENT 2			
		10 PFU		100 PFU		10 PFU		100 PFU	
		D/T	% dead	D/T	% dead	D/T	% dead	D/T	% dead
WNV	-	5/8	63	9/10	90	4/8	50	10/10	100
WNV	20	2/8	25	5/10	50	3/8	38	6/10	60
WNV	200	0/10	0	2/10	20*	0/8	0*	4/10	40*
WNV	1000	0/10	0	3/10	30*	1/8	13	5/10	50*
WNV+COLD	-	8/10	80	10/10	100	7/8	88	10/10	100
WNV+COLD	20	4/10	40*	5/10	50*	4/8	50	6/10	60
WNV+COLD	200	2/10	20*	4/10	40*	3/8	38	3/10	30*
WNV+COLD	1000	3/10	30	4/10	40*	2/8	25	4/10	40*

* $p<0.05$ as compared to non treated group (control).

20 mg./kg. in RSSP I.P. on day -1, 0, 2, 4, 6, 8.

200 mg./kg. in RSSP S.C. day -1, 3, 6.

1000 mg./kg. in P. oil S.C. day -1, 3, 6.

The effect of DHEA on virus levels in the brain.

In the following experiments we tested if there is a correlation between virus level in the brain and mortality rate. Groups of 8 mice were sacrificed 7 days after inoculation and the brain was removed. The data in table 4 shows that in non-stressed mice inoculated I.P. no virus was detected. In stress-exposed mice virus was detected in 75% of the brains, while in DHEA treated mice only 25% of the brains were virus-positive. Virus levels in the brain in virus-positive mice exposed to cold, isolation or dexamethasone injection resulted in 8.2, 7.7 and 7.4 $\log_{10}$ PFU, respectively (table 4). Whereas in I.C. injected mice virus was detected in all the brains with a mean level of 8.2 $\log_{10}$ PFU. DHEA treatment reduced brain virus levels on day 7 after inoculation to a mean of 5.9 $\log_{10}$ PFU with no significant difference related to the stress applied. The low levels are probably the result of the delayed course of the disease as expressed in the prolonged time of death. Furthermore, a similar tendency was found when virus levels were compared in blood and spleen of DHEA treated and non-treated mice. In all experiments tested, the level of virus in blood and spleen in DHEA treated mice was lower than in non-treated mice.

TABLE 4

Brain virus levels of mice 7 days after inoculation and exposure to various stress paradigms.

Treatment Group	DHEA	Brain Positive for Virus (%)	Titer of Virus $\log_{10}$ PFU/brain
WN-25	-	0	<2
	+	0	<2
WN-25+Cold	-	75	8.2±0.4
	+	30	6.0±0.4
WN-25+Isolation	–	80	7.7±0.3
	+	25*	5.9±0.4
WN-25+Dexamethasone	-	70	7.4±0.5
	+	20*	5.8±0.4
WN-25 (control I.C.)	-	100	8.4±0.4

* $p<0.05$ as compared to non treated group.

DHEA - 20 mg./kg. on days 1 and 0 before and on days 2, 4, 6 after virus inoculation.

Dexamethasone - 2 mg./kg., 2 hours before and on the day after inoculation.

DISCUSSION

The experiments described herein indicate that DHEA is effective in protecting mice from stress induced immunosuppression. This protection is demonstrated using WNV or attenuated noninvasive WN-25 encephalitis virus. DHEA treatment prevented stress-induction of encephalitis (WN-25), postponed the onset of disease and prevented the involution of spleen and thymus. The involution of lymphoid organs serves as a common parameter to assess the immunosuppresive effects of stress.

In the present study three stress paradigms were used to induced attenuated WNV (WN-25) encephalitis; cold stress (physical), isolation (social) and dexamethasone injection (pharmacological imitation of glucocorticoid response). DHEA treatment was found to be effective in all three paradigms; lymphoid organs involution was antagonized by DHEA treatment and weight reduction was arrested at near normal level in stress-exposed, infected and non-infected groups (Fig. 2 a,b). Moreover, DHEA was effective in preventing cold stress induced lymphoid organs' involution even after WNV inoculation (table 1). In WN-25 inoculated mice, DHEA treatment reduced mortality from 67%, 75% and 67% to 22%, 32% and 11% in cold, isolation or dexamethasone stress treatments respectively (Fig. 3). On the other hand, in WNV inoculated mice DHEA reduced mortality from 100% to 80%, 60% and 40% when applied at concentrations of 5, 10 and 20 mg./kg. respectively (table 2). In higher doses of DHEA the protective effect was more pronounced (table 3). However, no difference was found between 200 mg./kg. to 1,000 mg./kg.

The effect of DHEA on preventing mortality that was induced by various stress paradigms may imply that DHEA acts as a common antagonist to stress induced immunosuppression with no regards to the type of stress applied. Furthermore, the efficacy of DHEA in preventing dexamethasone induced encephalitis strongly supports its mechanism of action to be counteraction of glucocorticoids (GC).

DHEA was effective in preventing stress-induced and dexamethasone - induced lymphoid organs involution. This effect further supports its mechanism of action to be prevention of GC induced immunosuppression that occurs following application of different types of stress and through viral infection (Dunn et. al. 1989).

A adative of viral infection (WNV or WN-25) and the stress paradigms in lymphoid organs involution was observed. This combined effect was blocked by DHEA. That and DHEA's protective effect on a variety of lethal viral infections (Loria et. al. 1988, Ben-Nathan et. al 1991) may indicate that DHEA really acts as a common inhibitor to stress effects including virus - induced immunosuppression.

Recently DHEA was suggested to have an immunomodulating activity. It was found to prevent dexamethasone - induced thymic involution (May et. al. 1990), to increase IL-2 production (Daynes et. al. 1990) and to act as a "buffer hormone" (Regelson et. al.)
Questions are raised regarding DHEA's mechanism of action. The lack of in vitro antiviral activity (Loria et. al. 1988, Ben-Nathan et. al. 1991) and its inability to protect SCID (severe combined immunodeficiency) mice infected with the attenuated WN-25 virus (unpublished data) point to its effect on host resistance rather than on virus itself.

In conclusion, the present study provides direct evidence of the protecting effects of DHEA as "anti-stress" agent. Its ability to prevent mortality (against WNV or WN-25) and lymphoid organs involution caused by stress-induced immunosuppression supports the notion that its activity is based on the counteraction of glucocorticoids.

Therefore, additional studies should investigate the immunomodulating activity of DHEA on other types of viruses; to clarify the direct mechanism by which DHEA counteracts glucocorticoids effects and DHEA efficacy in treatment of infections and of stress adverse effects.

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