Naloxone Ameliorates the Pathophysiologic Changes Which Lead to and Attend an Acute Stroke in Stroke-Prone/SHR

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SUMMARY Stroke-prone, spontaneously hypertensive rats (SP/SHR) were fed a low protein (8%) fish diet + 1% saline at the time of weaning; some were treated with Naloxone (0.4 mg/100 gms bw/sc/2 × daily/5 days per week). Naloxone-treated animals did not develop high blood pressure or strokes. Sixty-two days after feeding the low protein fish diet, blood pressure levels reached 260–300 mmHg and all of the non-treated animals exhibited acute and severe strokes; Naloxone treatment was again initiated for half of the SP/SHR. By Day 4 (post stroke), all of the non-treated SP/SHR were dead; Naloxone-treated SP/SHR survived until Day 12 (post stroke). Naloxone treatment during the post-stroke period caused significant reduction of blood pressure, ACTH, and beta-endorphin levels concomitant with reduced cerebral edema and clearance of hepatic lipid infiltration. It is suggested that anti-opiate treatment may ameliorate the severe hypertension-inducing effects of a low protein fish diet and thereby prevent the appearance of strokes in SP/SHR as well as palliate the cerebral edema and fatty liver which characteristically appear in the immediate post-stroke period in fish-fed SP/SHR. The central mechanism of this palliative effect may be through reduced hypothalamic-pituitary-adrenal activity.

THE SPONTANEOUSLY HYPERTENSIVE AND STROKE-PRONE RAT (SP/SHR) DEVELOP MUCH MORE SEVERE HYPERTENSION THAN IS CUSTOMARILY FOUND IN THE SP/SHR STRAIN. THE MODE OF HYPERTENSION IS ASYMMETRICAL, WITH AN INITIAL PHASE OF PROGRESSIVE DEVELOPMENT OF HIGH BLOOD PRESSURE AND EVEN-TORWARD APPEARANCE OF STROKE IN SP/SHR.

In a genetic sub-strain, variant of the SP/SHR which spontaneously develops massive obesity as well as hypertension (Obese/SHR), adenalec-tomy, pituitary gland suppression with dexamethasone, or inhibition of hypothalamic neurotransmitters by the anti-opiate, naloxone, would obviate the development of the genetically-programmed massive obesity and hypertension by reducing hypothalamic-pituitary activity. The question arose whether suppression of the hypothalamic-pituitary-adrenal axis by naloxone would similarly prevent the pathogenesis of or ameliorate the untoward changes which attend the development of acute, spontaneously appearing strokes in low protein fish diet-fed SP/SHR.

Methods

All of the SP/SHR animals were offspring of breeder stock derived from the original SP/SHR strain of Kyoto, Japan, kindly provided by Dr. Carl T. Hansen, Animal Genetics Division, N.I.H. When the SP/SHR were weaned from their mothers (at 23 days of age), they were fed a low protein (8%) fish diet + 1% saline drinking water. The low protein fish diet was purchased from Funabashi Farms, Funabashi City, Chiba, Japan. Systolic blood pressure was measured every 10 days under light Seconal anesthesia using the Friedman: Freed micromephonic manometer and indirect tail cuff procedure. At 30 days of age (7 days post inception of feeding the fish diet), some of the animals were given 0.4 mg naloxone (Endo Laboratories Inc., Garden City, N.Y.). Naloxone is N-allyl-14-hydroxydihydro-norfentanyl, a narcotic or opiate antagonist. The hydrochloride powder was suspended in 0.9% saline and was administered at a dose level of 0.4 mg/100 gm bw/sc/2 × daily/5 days per week, to determine whether naloxone treatment would alter the usual progressive development of high blood pressure and eventual appearance of stroke in SP/SHR.

At 30 days of age and at 20 day intervals thereafter, 10 naloxone + fish diet-treated animals and 10 fish diet + no rx animals were selected randomly and killed by decapitation for determination of blood ACTH and beta-endorphin levels as a temporal index of alterations in hypothalamic-pituitary-adrenal activity. At 92 days of age (62 days post feeding the fish diet + 1% saline), the animals receiving the low protein fish diet + 1% saline began to have severe strokes; Naloxone treatment was again initiated for half of the treated animals exhibited acute and severe strokes; Naloxone treatment was again initiated for half of the treated animals. Stroked-prone/SHR. By Day 4 (post stroke), all of the non-treated SP/SHR were dead; Naloxone-treated SP/SHR survived until Day 12 (post stroke). Naloxone treatment during the post-stroke period caused significant reduction of blood pressure, ACTH, and beta-endorphin levels concomitant with reduced cerebral edema and clearance of hepatic lipid infiltration. It is suggested that anti-opiate treatment may ameliorate the severe hypertension-inducing effects of a low protein fish diet and thereby prevent the appearance of strokes in SP/SHR as well as palliate the cerebral edema and fatty liver which characteristically appear in the immediate post-stroke period in fish-fed SP/SHR. The central mechanism of this palliative effect may be through reduced hypothalamic-pituitary-adrenal activity.

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gms bw of naloxone sc/2 x daily in addition to the special diet. The introduction of naloxone at this point was to determine whether naloxone would palliate the untoward conditions which attend an acute stroke in fish-fed SP/SHR. Animals were kept under constant surveillance. Those that became moribund were killed by decapitation and their blood (truncal) was used for measurement of ACTH and beta-endorphin levels. Blood samples were taken by cardiac puncture of surviving animals at 2 day intervals for 12 days post-inception of stroke. The experiment was terminated at this time because of the greatly reduced number of survivors.

ACTH and beta-endorphin levels were measured using radioimmunoassay kits purchased from CIS Radiopharmaceuticals, Inc., Bedford, Mass. (for ACTH) and from Immuno Nuclear Corp., Stillwater, Minn. (for beta-endorphin). The brains, heart, and other key organs of each animal were examined for gross evidence of vascular related degenerative changes. Pertinent organs from each rat were fixed in 10% formalin, embedded in paraffin, sectioned at 3 μm, and stained with hematoxylin and eosin for microscopic examination. Statistical analysis of the data was performed using a one-way analysis of variance, chi square test, and Student’s t-test.

Results

General observations

The animals responded to the low protein fish diet + 1% saline exactly as they had in a previous experiment.1 Within two weeks, the animals appeared lean and growth retarded, but otherwise healthy and active. Precisely as before,1 62 days after the start of the fish diet, the animals began to convulse and leap about wildly in their cages. Minutes later, they manifested severe blanching of the eyes, a Horner’s syndrome-like condition, and bilateral or ipsilateral paralysis of the extremities with and without extensor rigidity. Within 3 to 4 days, all of the animals (n = 40) manifested signs of severe cerebral damage, i.e., stroke, and 30 animals survived this initial stroke. Most of the animals not treated with naloxone (n = 15) were moribund (n = 11) on Day 2 post the initial appearance of stroke and had to be killed. The remainder (n = 4) had to be killed by Day 4 because they too were moribund (fig. 1). Treatment with naloxone had a remarkable life-sustaining effect. The naloxone-treated animals (n = 15) moved about despite their paralysis, ate, and appeared to be recuperating for the first 7 days post-stroke. With time, despite continuous treatment with naloxone, the condition of the surviving animals began to deteriorate. By Day 10 the survivors (n = 7) became prostrate and by Day 12, the survivors (n = 3) were moribund and had to be killed (fig. 1).

Blood pressure

The blood pressure of all of the fish diet-fed SP/SHR rose rapidly. At 50 days of age, there was a definite break in the ascent of the blood pressure of the naloxone-treated SP/SHR reaching a level of 160 mmHg at 90 days of age (fig. 1). (Blood pressure levels over 130 mmHg are considered to be abnormal in the rat.) In contrast, the blood pressure of the non-treated, fish diet-fed animals rose exponentially reaching levels of 260 mmHg and above at 90 days of age (fig. 1). When the animals began having acute strokes, the blood pressure of the non-treated SP/SHR continued to rise approaching 300 mmHg prior to death (fig. 1). SP/SHR treated with naloxone at the time when acute strokes first appeared manifested definite and progressive reduction of their blood pressure (fig. 1).

Adrenocorticotropic hormone (ACTH)

The feeding of the fish diet + 1% saline was associated with considerably increased secretion of ACTH approaching levels of 800 pg/ml (fig. 2). (The blood ACTH levels of intact 30 day old SP/SHR ranges between 300—400 pg/ml). At the outset, treatment with naloxone did not appear to reduce ACTH secretion. With time, however, naloxone treatment caused a significant (p < 0.001) decrease in ACTH secretion compared to progressive and marked increases in blood ACTH levels in SP/SHR fed the low protein fish diet (fig. 2).

In the post-stroke period circulating ACTH levels...
were exceedingly high, i.e., rising from 140 pg/ml (fig. 2) to 3000 pg/ml (fig. 3). Again, the ACTH levels of the post-stroke, naloxone-treated animals were significantly ($p < 0.001$) lower than their cohorts fed the fish diet only.

**Beta-endorphin**

The pattern of change in circulating beta-endorphin levels paralleled those obtained for ACTH (figs. 2 and 3). Naloxone treatment had little effect on beta-endorphin levels during the early pre-stroke stages of the experiment. However, beta endorphin levels were definitely reduced ($p < 0.001$) after chronic naloxone treatment in the pre-stroke period (fig. 4), whereas the beta-endorphin levels of the non-treated, diet-fed animals rose progressively.

The changes in beta-endorphin levels in the post-stroke period resembled the changes seen in ACTH levels at the same time (figs. 2 and 3). Beta-endorphin levels did not manifest the greatly increased secretion shown by ACTH in the post-stroke period (cf. figs. 3 and 5). Naloxone-treated SP/SHR manifested significant reduction ($p < 0.001$) of their beta-endorphin levels (fig. 5).

**Pathology**

At necropsy, large ipsilateral mixed hemorrhagic and thrombotic space occupying lesions of the parietal lobe were found with remarkable consistency (severity, morphology, etc.) in all SP/SHR. The surrounding brain tissue was severely edematous and swollen. (Details of the gross and microscopic pathology of these fish diet-induced strokes have been published.)

**Fig. 2** Changes in blood ACTH levels of SP/SHR fed a low protein fish diet and 1% saline from the time of weaning until young adulthood and prior to the appearance of strokes. Some of the animals were treated with naloxone during this same period. The height of each column depicts the Mean ± Standard Error; $n = 10$. The same protocol applies to figure 4.

**Fig. 3** Changes in blood ACTH levels in SP/SHR which developed acute strokes following chronic feeding of a low protein fish diet + 1% saline. Some of the animals were treated with naloxone immediately after the appearance of stroke. The height of each bar depicts the Mean ± Standard Error; ( ) = number of samples. The same protocol applies to figure 5.

though the hemorrhagic infarcts in the naloxone-treated SP/SHR appeared to be identical to the lesions found in the non-treated animals, there was a striking absence of swelling and edema (as demonstrated by gross and microscopic examination) in the vital brain tissue immediately surrounding the lesion in the involved hemisphere and in the contralateral hemisphere as well. The hepatic parenchyma of the non-treated SP/SHR was severely infiltrated with lipid while the naloxone-treated animals did not show any fatty infiltration of the liver despite the fact that lipid infiltration is characteristic of the SP/SHR sub-strain irrespective of treatment or dietary manipulation.
These findings are all the more impressive in light of the fact that all of the fish-fed animals, treated and non-treated, displayed unilateral, massive hemorrhagic infarcts of the parietal lobe of uniform size and severity, both by gross and microscopic examination. Until Day 10 post stroke, the naloxone-treated animals were active, eating, drinking, and apparently recovering from gross motor impairment vs their non-treated moribund brothers who were all dead by Day 3 post-stroke. One outstanding feature of the post-stroke, naloxone-treated animals was their apparently normal cardiorespiratory behavior vs the intermittent and deep respirations displayed by non-treated SP/SHR. Endorphins are known to depress medullary respiratory centers and high doses of naloxone will greatly improve respiratory function. Several investigators have noted that naloxone will alleviate hemiparesis and shock during the acute post-stroke period. Perhaps the most potent palliative effect exhibited by naloxone was the virtually complete clearance of grossly-discernible cerebral edema. In addition, the effective clearance of hepatic lipid by naloxone may have contributed to the superior survival of the animals. Fat infiltrating the hepatic parenchyma would greatly interfere with metabolism and conjugation of adrenal steroids creating a state of relative hyperadrenocorticism which compromises rather than aids survival. SP/SHR afflicted by acute cerebral damage marshall excessive amounts of ACTH, adrenal steroids, and endorphins, which may cause cerebral edema and hepatic lipid accumulation. By alleviating the detrimental excess of ACTH, adrenal steroids, and endorphins, treatment with naloxone would improve survival.

Some investigators are dubious of the salutary effects of naloxone and question whether endorphins are truly involved in the body’s response to cerebral ischemia. The latter do agree that differences in the dose of naloxone given, time of inception of treatment, and the experimental model used could account for their lack of enthusiasm toward naloxone in the treatment of neurologic deficit. The positive salutary findings described in the experiment herein could also be ascribed to the fact that: 1) the dose of naloxone used had previously proven to be successful in preventing the development of spontaneous obesity in Obese/SHR (to be published), 2) it had been determined that naloxone should be administered several times during a 24 hour period because it is rapidly metabolized, and 3) naloxone treatment was initiated within 48 hours of the inception of stroke. The post-stroke animals were not handled except those injected with naloxone. Some investigators suggest that handling of animals during the post-stroke period causes improvement in the animal’s neurologic condition. Handling could have a salutary effect if it is done frequently. However, the author doubts whether handling of these animals to inject naloxone (2 x daily) could account for the striking salutary changes observed.

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**References**

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