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 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.

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THE SPONTANEOUSLY HYPERTENSIVE AND stroke-prone rat (SP/SHR) will develop much more severe high blood pressure if it is fed a low protein fish diet + 1% saline vs a low protein diet derived from animal tissue. The regimen of feeding a fish diet + 1% saline will cause severe, hemorrhagic infarcts in the parietal lobe appearing in virtually all animals after 90 days on this regimen. These experimentally-induced strokes are of particular interest because their pathogenesis and morphologic characteristics closely resemble clinical aspects of acute stroke, e.g., involving blood pressure levels, the anatomical distribution of the middle cerebral artery, and destruction of the basal ganglia. In prior experiments utilizing the fish diet to produce strokes in SP/SHR, the author found that this dietary regimen caused marked stimulation of the pituitary-adrenal axis, i.e., increased adrenocorticotropic hormone (ACTH) and beta-endorphin secretion, as well as hyperlipidemia, and hyperglycemia. In a genetic sub-strain, variant of the SP/SHR which spontaneously develops massive obesity as well as hypertension (Obese/SHR), adrenalectomy, 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, Funabshi City, Chiba, Japan. Systolic blood pressure was measured every 10 days under light Seconal anesthesia using the Friedman: Freed microphonic 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-hydroxydihydromorphone, 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. Within 48 hours of the appearance of strokes, 30 surviving SP/SHR were selected randomly and half (n = 15) continued to receive the fish diet + 1% saline while the other half (n = 15) were given 0.4 mg/100

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fig. 1 Changes in systolic blood pressure by Stroke-Prone SHR which were fed an 8% low protein diet derived from fish and 1% saline to drink from the time they were weaned and during the active growth spurt of young adulthood, i.e., until 90 days of age. Some of the SP/SHR were given naloxone during this same period. The naloxone treated animals showed a plateau in their rapidly rising blood pressure. At 90 ± 2 days, all of the animals began having strokes. The blood pressure rose inexorably in animals fed the fish diet only and were all dead 4 days post stroke. SP/SHR given naloxone as soon as acute strokes appeared exhibited progressive reduction of their blood pressure and superior survival. Each point represents the Mean ± Standard Error; numbers enclosed ( ) indicate the number of samples.

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). The 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.)

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.

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.

FIG. 4 Changes in blood levels of immunoreactively detectable beta-endorphin levels prior to stroke.
These findings are all the more impressive in light of cause the significant prolongation of survival and re-
activation of fish-fed animals, treated and non-
treated, displayed unilateral, massive hemorrhagic in-
facts of the parietal lobe of uniform size and severity,
Naloxone ameliorates the pathophysiologic changes which lead to and attend an acute stroke in stroke-prone/SHR.

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