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) 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.1 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.1,2 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.1,3

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 adrenocorticotrophin (ACTH) and beta-endorphin secretion, as well as hyperlipidemia, and hyperglycemia.1,3 In a genetic sub-strain, variant of the SP/SHR which spontaneously develops massive obesity as well as hypertension (Obese/SHR), adrenalectomy,4 pituitary gland suppression with dexamethasone,5 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 Funahashi Farms, Funabashi 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-hydroxydihydro-nor-morphinone, 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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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

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.
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Discussion

One of the outstanding features of the pharmacodynamics of naloxone is that this drug may be used both as a therapeutic or diagnostic agent. This pharmacodynamic attribute of naloxone has been used to ferret out a great deal of information implicating the hypothalamic-pituitary-adrenal axis and endogenous opioid production, e.g., enkephalins, beta-endorphins, etc., as playing key roles in shock, trauma, and cerebrovascular disease in animals and in man.1-12 Naloxone is not effective in hypophysectomized animals.13 Adrenalectomy is followed by a compensatory increased release of ACTH and beta-endorphin.14 Adrenalectomy also potentiates the lethal effects of intravenous beta-endorphin.15 All of the foregoing can be reversed by treatment with glucocorticoids.14 Therefore, endogenous opioids are secreted in conjunction with stress-induced glucocorticoid production. The author noted that all SP/SHR subjected to the dietary regimen of a low protein-fish diet + 1% saline manifested a considerable increase in blood ACTH and beta-endorphin levels.1,4 That is, the modus operandi of the low protein fish diet + 1% saline could be through its ability to evoke the stress reaction.1,4

The unusually rapid ascent of blood pressure concomitant with progressively increased secretion of ACTH and beta-endorphin in fish-fed SP/SHR strengthened the likelihood that the low protein fish diet evoked stimulation of the hypothalamic-pituitary-adrenal system.1,4 It should be emphasized that when SP/SHR were fed a low protein diet derived from animal tissue, they did not develop strokes, high blood pressure, or increased secretion of ACTH.1

It is not clear what specific physiologic mechanisms were entrained by the administration of naloxone to cause the significant prolongation of survival and remarkable palliation of the acute post-stroke condition. 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.16,17 Several investigators have noted that naloxone will alleviate hemiparesis18 and shock19 during the acute post-stroke period. Perhaps the most potent palliative effect exhibited by naloxone was the virtually complete clearance of grossly-discernible cerebroedema. 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.20,21 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.22-24 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.22-24 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 been 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.22-24 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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