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Dehydration and Stress Do Not Explain Severe Weight Loss After Experimental Stroke in Rats

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Abstract: Transient middle cerebral artery occlusion (MCAO) in rats is perhaps the most common experimental stroke model. MCAO in rats is associated with a severe loss of body weight of unknown etiology. The purpose of the present study was to investigate whether dehydration, stress, or altered hormonal secretion contribute to weight loss after MCAO. The right middle cerebral artery of male Wistar rats was occluded for 120 min using the intraluminal filament method. Sham-operated rats were used as controls. Body weight and sensorimotor deficit were measured after surgery. Rats were killed 3 and 12 h, and 1, 2, 3, 7, 14, and 28 d after surgery and blood samples were obtained by cardiac puncture. Plasma osmolality, corticosterone, leptin, and TSH, all of which might be associated with loss of body weight, were measured. There were no differences in plasma osmolality, corticosterone, or TSH between ischemic and sham-operated rats after surgery or were only transiently elevated in ischemic rats. Plasma leptin decreased in ischemic rats on postoperative days 1, 3, 7, and 28. These data suggest that weight loss after MCAO in rats is not associated with dehydration or long-lasting postoperative stress.

Key words: Dehydration, explain severe, experimental stroke and rats

Introduction

Valid experimental stroke models are needed for preclinical drug testing and for studies related to the pathophysiology of stroke or recovery processes following stroke. Transient occlusion of the middle cerebral artery (MCAO) in rats using the intraluminal filament is perhaps the most widely used experimental model due to its similarity to stroke (Longa *et al.*, 1989). The middle cerebral artery is the most commonly affected vessel in stroke (Mohr *et al.*, 1986), and reperfusion occurs in up to 50% of stroke patients (Saito *et al.*, 1987). In addition, the model is relatively noninvasive, because craniectomy is not necessary. The disadvantages are numerous, however, including inadequate MCAO, subarachnoid hemorrhage, intraluminal thrombus formation, and severe weight loss after surgery (Schmid-Elsaesser *et al.*, 1998).

The weight loss in rats subjected to transient MCAO is important, as it might affect behavioral performance of rats, particularly in food rewarded tasks (Yamamoto *et al.*, 1988; Rogers *et al.*, 1997; Reglödi *et al.*, 2003). Yamamoto *et al.* (1988) reported a significant decrease in body weight (~35 g) at 1 week after MCAO, followed by gradual recovery to preoperative levels. Menzies *et al.* (1992) reported that the rat body weight decreased by 7.5 to 12.6 % during the first 24 to 48 h after MCAO. Weight decline continued until postoperative day 6 (17.4 %) and rats returned to the preoperative weight by postoperative day 11. In our hands, the weight loss is typically 15 to 20 % after MCAO despite administration of supplemental 0.9 % NaCl (i.p.). The purpose of the present study was to investigate whether dehydration, long-lasting postoperative stress, or altered hormonal secretion contribute to the weight loss after transient MCAO in rats.

Materials and Methods

Animals: Adult male Wistar rats (HsdBrl:WH, Hannover origin; National Laboratory Animal Centre, Kuopio, Finland) weighing 250 to 350 g at the beginning of experiment, were used in the present study. Animals were housed individually in standard cages (53 cm x 32.5 cm x 20cm) in a temperature- (20±1°C) and light-controlled (12h:12h) environment. Food and water were available *ad libitum*. Experimental procedures were conducted in accordance with the European Community Council directives 86/609/EEC and were approved by the Committee for the Welfare of Laboratory Animals of the University of Kuopio and by the Provincial Government of Kuopio.

Middle Cerebral Artery Occlusion: Transient middle cerebral artery occlusion was induced

using the intraluminal filament technique (Longa *et al.*, 1989). Rats were anesthetized using 3 % halothane in 30 % O_2 and 70 % N_2O. Anesthesia was maintained throughout the operation with 0.5 to 1 % halothane delivered through a nose mask. The right common carotid artery was exposed through a midline ventral cervical incision under a surgical microscope and carefully separated from the adjacent sympathetic nerves. The right common carotid artery and the internal carotid artery were then clamped with microvascular clips to prevent bleeding during insertion of the filament. The external common carotid artery was ligated distally with a nylon suture, cut with microscissors, and electrocoagulated. A heparinized nylon filament (⌀ 0.25 mm, rounded tip) was inserted into the stump of the external common carotid artery. The filament was advanced 1.9 to 2.1 cm into the internal common carotid artery, to occlude blood flow to the MCA territory, until resistance was felt. The filament was held in place by tightening the suture around the internal common carotid artery and placing a microvascular clip around the artery. Body temperature was monitored and maintained at 37°C using a thermoregulatory heating pad connected to a rectal probe (Harvard Homeothermic Blanket System, Harvard Apparatus, Holliston, MA). The filament was removed after 120 min to allow reperfusion in the vascular territory of the MCA. The stump of the external carotid artery was electrocoagulated followed by removal of the common carotid artery clip. The cervical incision was closed with nylon sutures and the animals were returned to their home cages. Sham-operated rats were treated in a similar manner except the filament was not introduced into the internal common carotid artery. Animals were given supplemental 0.9 % NaCl (i.p., 5 ml) at the end of the operation and as necessary postoperatively.

Assessment of Sensorimotor Deficit: The limb-placing test was a modified version of a test described by de Ryck *et al.* (1989). The test was used to study sensorimotor deficits 3 and 12 h, and 1, 2, 3, 7, 14, and 28 d after surgery, and to assign rats to behaviorally equivalent groups (Jolkkonen *et al.*, 2000; Puurunen *et al.*, 2001a). The rats were habituated for handling and testing before inducing ischemia. The test consisted of seven tasks, which were scored as follows: 2 points, if the rat performed normally; 1 point, if the rat performed with a delay of more than 2 s and/or incompletely; 0 points, if the rat did not perform normally. Both sides of the body were tested. The maximum possible score achieved by the sham-operated rats was 14. Only the rats with total scores of less than 10 were included into ischemic groups.

Plasma Osmolality and Hormone Measurements: Rats were killed 3 and 12 h, and 1, 2, 3, 7, 14, and 28 d after operation at the same time of day (1200-1400 h). Rats were anesthetized with CO_2 and blood samples were taken by cardiac puncture using heparinized needles. Samples were centrifuged (4 °C, 15 min, 2500 rpm) and then plasma was divided into 250- µl aliquots to be stored at -80°C until analyzed.

Plasma osmolality was analyzed to determine the level of dehydration, based on the freezing point depression using a supercooling method (Osmostat OM-6020). This measures the changes in the freezing point that occur in solutions with increasing osmolality. Plasma corticosterone was measured using a commercial radioimmunoassay RIA kit (Corticosterone ^{125}I RIA Kit, for rats and mice, ICN Biomedicals) as an index of the general response to stress. Plasma leptin was analyzed using a commercial RIA kit (Leptin ^{125}I-Rat RIA Kit, for rats, Linco Research, Inc.) to measure the regulation of food consumption. Plasma levels of TSH were measured using a commercial RIA kit (rTSH ^{125}I assay system with magnetic separation, Amersham Pharmacia Biotech) to investigate thyroid function and, indirectly, cellular metabolic activity.

Statistics: All data were analyzed using SPSS for Windows. Limb-placing test scores were analyzed using the Mann-Whitney U test. Body weight, plasma osmolality, corticosterone, leptin, and TSH levels were analyzed using a two-tailed independent samples t-test. A P-value of less than 0.05 was considered to be statistically significant.

Results

Body Weight: The body weight of the ischemic rats decreased approximately 16.7 % after 24 h and 20.4 % after 48 h following MCAO. Body weight continued to decline until postoperative day 7 (20.8 %), followed by a return to preoperative levels by the end of the follow-up period. Body weight was statistically different between ischemic and sham-operated rats at all postoperative time points (*$P<0.05$; Fig. 1).

Sensorimotor Deficit: Sensorimotor deficit as assessed by the limb-placing test was

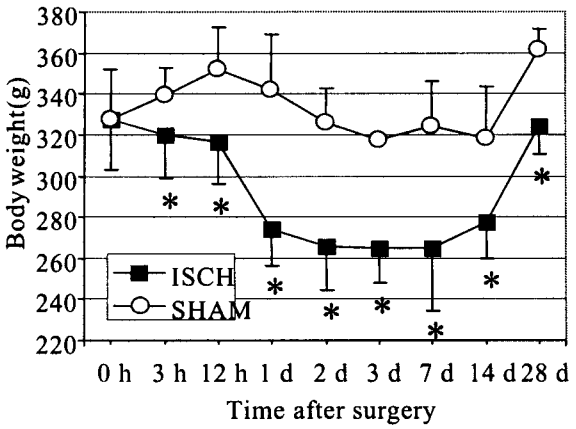

Fig. 1: Body weight of rats subjected to transient middle cerebral artery occlusion (MCAO). Body weight was statistically different between ischemic (ISCH) and sham-operated (SHAM) rats from 3 h to 28 d after operation (*P<0.05).

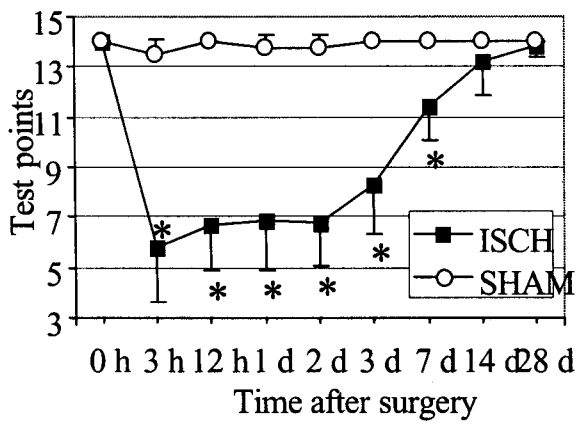

Fig. 2: Sensorimotor deficit of rats subjected to transient middle cerebral artery occlusion (MCAO). There was a statistically significant difference between ischemic (ISCH) and sham-operated (SHAM) rats from 3 h to 7 d after operation (*P<0.05).

significantly different between the ischemic and sham-operated rats 3 and 12 h, 1, 2, 3 and 7 d after surgery (*P<0.05; Fig. 2).

Plasma Osmolality and Hormones: Plasma osmolality was statistically different between ischemic and sham-operated rats at 12 h and 14 d after surgery (*P<0.05; Fig. 3). Plasma

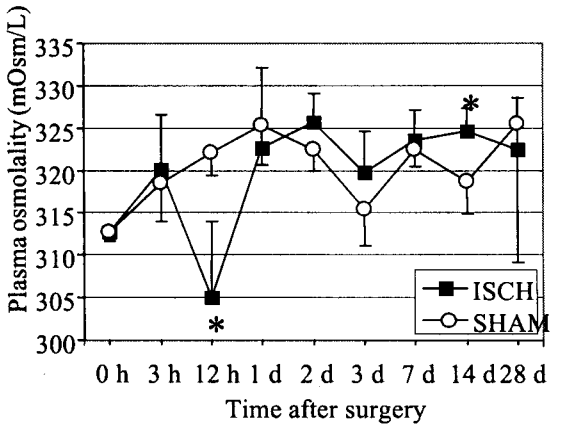

Fig. 3: Plasma osmolality of rats subjected to transient middle cerebral artery occlusion (MCAO). Osmolality was statistically different between ischemic (ISCH) and sham-operated (SHAM) rats only at 12 h and 14 d after operation (*P<0.05).

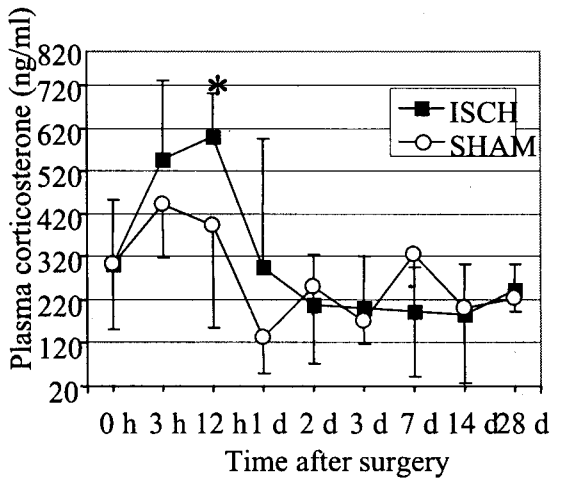

Fig. 4: Plasma corticosterone of rats subjected to transient middle cerebral artery occlusion (MCAO). Corticosterone levels were statistically different between ischemic (ISCH) and sham-operated (SHAM) rats only at 12 h after operation (*P<0.05).

corticosterone levels were statistically different between ischemic and sham-operated rats at 12 h after surgery (*P<0.05; Fig. 4). Plasma leptin levels were statistically different between ischemic and sham-operated rats on postoperative days 1, 3, 7, and 28 (*P<0.05; Fig. 5). Plasma TSH levels were not statistically

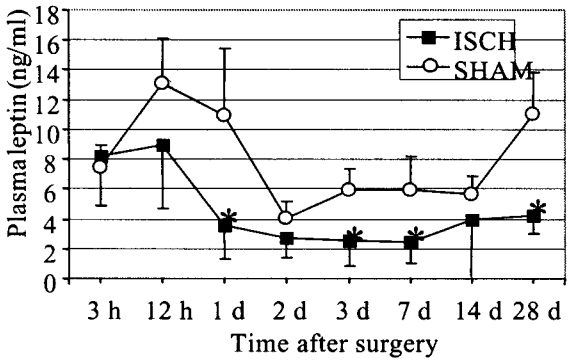

Fig. 5: Plasma leptin of rats subjected to transient middle cerebral artery occlusion (MCAO). Leptin levels were statistically different between ischemic (ISCH) and sham-operated (SHAM) rats at 1, 3, 7 and 28 d after operation (*P<0.05).

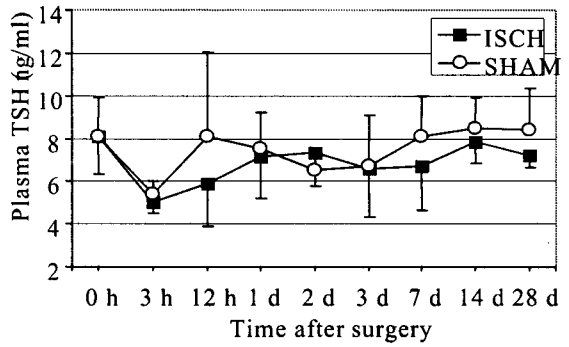

Fig. 6: Plasma TSH of rats subjected to transient middle cerebral artery occlusion (MCAO). There were no statistically significant differences between ischemic (ISCH) and sham-operated (SHAM) rats.

different between ischemic and sham-operated rats at the time points measured (Fig. 6).

Discussion

The present study suggests that weight loss, a typical complication after MCAO in rats, is not associated with dehydration, long-lasting postoperative stress, or altered hormonal secretion. Extensive corticostriatal damage of rats subjected to MCAO, resulting in difficulty eating and swallowing, is a more likely explanation for weight loss, which is supported by a previous study by Petullo *et al.* (1999). Also, Modo *et al.* (2000) reported that MCAO animals have reduced food intake for a prolonged period. The MCAO rats lost an average of 20.8 % of their preoperative weight over the first 7 d after surgery. Interestingly, the performance of ischemic rats in the limb-placing test was impaired at the same time points followed by a gradual, spontaneous recovery, consistent with previous results (Jolkkonen *et al.*, 2000; Puurunen *et al.*, 2001a; Puurunen *et al.*, 2001b; Karhunen *et al.*, 2003). Loss of body weight is not necessarily causally related to impaired performance in the limb-placing test, but likely confounds the performance of rats in food-rewarded tasks such as Montoya's staircase test.

Plasma osmolality is one of the most important physiologic variables used to determine the status of body dehydration. Increased plasma osmolality relates to reactive hyponatremia due to excess solute pulling water out of the cells, which eventually causes dehydration of the body. For example, Sebaal *et al.* (2002) reported that dehydration increased plasma osmolality in rats. Based on plasma osmolality measurement, however, the ischemic rats in the present study were not dehydrated. This might be due to the supplemental 0.9 % NaCl given to the rats.

Corticosterone is the principle glucocorticoid secreted by the adrenal glands (Shimizu *et al.*, 1983). Secretion of corticosterone is modulated by a complex negative feedback mechanism involving the central nervous system, hypothalamus, pituitary, and adrenals (Rattner *et al.*, 1980). Corticosterone measurements are a useful index of the general neuroendocrine response to stress. Corticosterone levels rise sharply following exposure to experimental stimuli such as drugs (Shimizu *et al.*, 1983), barometric shock (Rattner *et al.*, 1980), experimental disease state (Rattner *et al.*, 1979), or abrupt temperature shifts (Shimizu *et al.*, 1983). Thus, it was surprising that corticosterone levels were not increased in rats subjected to transient MCAO. This indicates that ischemic rats were not stressed due to extensive surgical procedures and that stress does not contribute to weight loss.

Leptin is a protein produced by the obesity gene. Adipose tissue produces leptin and releases it into the bloodstream. Leptin controls food consumption via central mechanisms and increases physical activity (Franceschini *et al.*, 2001; Sandoval *et al.*, 2003). In the present study, loss of body weight correlated with low plasma leptin levels. The immediate decrease in plasma leptin suggests, that this is a compensatory response that protects the body from starvation rather than the reason for body

weight loss.

Thyroid stimulating hormone (TSH) is a glycoprotein hormone with a molecular weight of approximately 28000 that is synthesized and secreted by basophil cells of the anterior pituitary. Release of TSH is controlled by hypothalamic thyrotropin releasing hormone. TSH stimulates synthesis and release of the thyroid hormones, thyroxine and triiodothyronine, which in turn control cellular metabolic activity (Szkudlinski *et al.*, 2001). Unilateral damage to the hypothalamus is typical in MCAO rats (He *et al.*, 1999), but the present study suggests that this does not affect plasma TSH levels. Thus, it is unlikely that TSH- mediated alterations in cellular metabolic activity account for the loss of body weight.

There is a need for continuous development of stroke models to provide more accurate and clinically relevant data, but also to ensure the general welfare of experimental animals. To overcome weight loss of rats subjected to MCAO, we recommend 1) careful monitoring of postoperative weight, 2) supplemental 0.9 % NaCl (i.p. or p.o.) to avoid dehydration, and 3) availability of liquid food or wet food pellets in home cages.

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