Properties of the sarcoplasmic proteins of red and white muscles of cat fish, *Clarias batrachus*

	Red muscle $\bar{x} \pm SD$	White muscle $\bar{x} \pm SD$	t	p
Calcium binding capacity (μmole Ca/mg protein)	0.92 ± 0.06	1.70 ± 0.06	23.73	0.01
Percentage water content	78.48 ± 2.43	82.09 ± 1.86	2.885	0.02
Calcium precipitated proteins (mg Ca bound protein/mg protein)	0.56 ± 0.09	0.34 ± 0.05	5.348	0.01
Ca-insensitive proteins (mg protein/mg total protein)	0.48 ± 0.09	0.61 ± 0.097	2.380	0.05
Ionic calcium (μg Ca/mg protein)	0.15 ± 0.03	0.32 ± 0.09	7.291	0.01
Ash content (percent wet weight)	1.66 ± 0.05	1.27 ± 0.05	1.879	0.2
Total calcium (μmole Ca/g dry weight)	10.80 ± 4.96	8.80 ± 2.59	0.875	0.2

Values are $\bar{x} \pm SD$ of 6; t = Student's t-test.

estimating the water loss when the wet tissue (2 g) was dried to constant weight at 105 °C. The dried muscle slices were ashed in porcelain crucibles in a muffle furnace at 650 °C and weighed to compute the ash content. A single pan electric balance (Mechaniki, made in Poland) was employed for these studies. 2 g of fresh muscle was homogenized in 10 ml redistilled water using a Potter-Elvinjin-homogenizer. A clear homogenate was obtained after centrifugation at 6000 × g for 20 min, and it dialized using a cellophane tube 15 cm long × 0.5 cm diameter against 20 ml water for 24 h at 5 °C. The Ca content of the dialysate was estimated by complexiметric method[5].

Sarcoplasmic proteins were extracted into 0.47 M KCl pH 7.0 according to Barany et al.[3]. Calcium binding proteins of them were estimated according to Weller[6]. Ca^{++} levels in the homogenate was estimated flame photometrically after analyzing the dialysate obtained after equilibrium dialysis[6]. The proteins of the homogenate was precipitated by addition of equal volume of 0.1 M Calcium acetate. The precipitate was collected by centrifugation at 600 × g for 20 min and the protein content of it was estimated colorimetrically[7]. The sarcoplasmic proteins in a 5 ml of the homogenate were precipitated with 10% trichloroacetic acid (TCA) and the precipitate was ashed in the muffle furnace at 650 °C. The calcium content of the ash was estimated by complexiметric method[5]. The TCA precipitated proteins in the same volume were estimated colorimetrically[7] to express the amount of bound calcium to unit weight of protein. The results were presented in the table.

The white muscle is characterized by greater water content and ionic calcium than the red muscle. The latter contains more Ca-precipitated proteins. Calcium insensitive (for charge neutra-lization at pH 7.0) proteins occur in greater concentration in white muscles. The muscles do not differ in total calcium and ash contents. Ca-binding capacity is more in white muscle, whereas calcium sensitive proteins are more in red muscle. The occurrence of high Ca binding capacity and rich Ca^{++} and a low Ca bound protein content of the white muscles corroborate the findings of Sreter[2] in the *Vastus lateralis* muscle of rabbit. Our results on Ca^{++} content in red and white muscles are not in agreement with those of Beecher et al.[8] who reported that the muscles do not differ in Ca levels. Another interesting observation is that the total Ca in the muscles do not vary but the availability of Ca in ionic or bound form varies with reference to the muscle.

The occurrence of more Ca-precipitated proteins (table) in the red muscle suggests the latter's low affinity to Ca^{++}. This affinity is mainly an electrostatic attraction between the protein and Ca^{++}[9]. These attractions have immense significance in the contractility of the muscle fibre[10].

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Proposed effects of brain noradrenaline on neuronal activity and cerebral blood flow during REM sleep

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Summary. We propose that the observed increases of both neuronal activity and cerebral blood flow seen throughout the brain during REM sleep may be effects of decreased central noradrenaline release.

Several groups of neuronal cell bodies located in the mammalian brainstem have been shown to contain[1] and to synthesize[2] noradrenaline (NA); the most well-known of these cell groups is the locus coeruleus. The axons of some of these NA-containing cells ramify widely throughout the spinal cord, brainstem, cerebellum and forebrain[3–10]. Pharmacological studies suggest that increased release of NA from axon terminals is coupled to increased discharge rates of the parent brainstem cells[11–15]. Central NA's precise functional role in central nervous system (CNS) is uncertain. Recent evidence, described below, suggests that central NA may affect both neuronal firing rates and cerebral blood flow (CBF) in regions innervated by NA-containing axons. We wish to propose here that the increases of both neuronal firing rates and CBF observed throughout the brain during rapid-eye-movement (REM) sleep are the result of decreased central NA release.

Recent experiments suggest that central NA may influence both neuronal firing rates and CBF. Stimulation of

the locus coeruleus inhibits the firing rates of cerebellar Purkinje[16], hippocampal[17], and spinal trigeminal nucleus[18] neurons. This effect is reproduced by microiontophoresis of NA onto these cells[18-20]. A central noradrenergic influence on CBF has recently been suggested[21]. Apparent innervation of the brain microvasculature by central noradrenergic fibres (persistent innervation after cervical sympathectomy) has been documented[22-24], and exogenous NA can cause cerebral vasoconstriction[25-27] and decreased blood flow[28]. We would expect that increased release of endogenous NA would result in vasoconstriction of the brain microvasculature, in the same way that increased release of peripheral NA from the sympathetic nervous system results in systemic vasoconstriction. Stimulation of the locus coeruleus pharmacologically has been shown to decrease CBF, whereas intraventricular injection of phentolamine, a noradrenergic alpha-blocker, increases CBF[21]. We would predict that increased CBF would result also from lesions of the locus coeruleus. Finally, since CBF and brain glucose consumption are positively correlated and believed to be coupled[29,30], lesions of the locus coeruleus might also be expected to increase brain glucose consumption. Preliminary data suggest that this may be true[31].

These experimental observations have suggested to us that activation of the diffusely-projecting brainstem NA-containing neurons, by liberating NA from nerve terminals throughout the brain, might result in decreased neuronal firing rates and decreased CBF. Conversely, lowered discharge rates of these brainstem NA-containing neurons, by decreasing NA release, would result in increased neuronal firing rates and increased CBF.

We believe that one consequence of this scheme is that some of the data on the physiology of sleep can be unified. It is known that CBF increases moderately during slow-wave sleep by approximately 15% over control awake subjects[32-35], and it increases greatly during REM sleep by approximately 80% over controls[33-37]. According to our proposal, such an increased CBF should be correlated with decreased central NA release, i.e. with decreased locus coeruleus discharge rates. In fact, locus coeruleus discharge rates have been recorded during sleep in the cat. The cat locus coeruleus is anatomically heterogenous, and within it is a specific group of NA-containing neurons which give rise to the ascending and cerebellar NA pathways[38,39]. Discharge rates of these neurons decrease moderately during the transition from wakefulness to slow-wave sleep, and they decrease drastically during REM sleep, many units having their lowest recorded discharge rates during this period[40,41]. From this nadir, discharge rates start to increase again during the last quarter of REM sleep, just before the animal awakens[42]. Several previous workers have suggested that the large increase of CBF seen during REM sleep was neurogenically mediated[34,36,37]. Also according to our proposal, lowered locus coeruleus discharge rates, by decreasing release of central NA, should result in increased neuronal firing rates in areas innervated by NA. In fact, during REM sleep neuronal activity does increase throughout the brain, and neuronal firing rates in cerebral cortex[43-45], thalamus[46], hippocampus[47], hypothalamus[48], and brainstem[49,50] are all higher than during wakefulness or slow-wave sleep.

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Pharmacological evidence is compatible with the suggestion that decreased central NA release is associated with REM sleep. In cats, inhibition of NA synthesis[51-53] or specific lesions of NA pathways[54] increase REM sleep time. In rats, the same results have been reported[55,56], whereas intraventricular administration of NA itself decreases REM sleep time[57].

We do not know how central NA exerts its widespread effects on neuronal firing rates and CBF. However, it is known that increases of regional CBF do occur during increased cerebral functional activity[58-62]. Likewise, increases of regional brain oxidative metabolism occur during increased brain functional activity[30,63-67], and it is believed that the activity-related increases of CBF serve to meet the increased energy demand incurred by elevated brain functional activity. It is possible, therefore, that observed noradrenergic effects on CBF are only secondary to the alterations of neuronal activity caused by a primary noradrenergic modulation of firing rates. If this were so, NA would act as presumably many neurotransmitters do, except that it would do so throughout the brain.

An alternative possibility is that central NA exerts primary control over CBF, neuronal firing rates, and perhaps even over oxidative metabolism. Brain is dependent on the continuous provision of both glucose and oxygen for oxidative metabolism[68], and it cannot sustain itself by anaerobic glycolysis[68] or by mobilization of glycogen stores[69-70]. A primary noradrenergic control of brain glucose consumption and CBF colud serve as a safety factor to increase oxidative metabolism quickly, protecting the brain from an energy debt for the first few moments after sudden changes in functional activity[71]. If this possibility were true, central NA might be an unusual neurotransmitter[72].

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72 Note added in proof: Recently electrical stimulation of the locus coeruleus has also been shown to decrease CBF [J. C. de la Torre, Neuroscience *1*, 455 (1976)].

Influence of metiamide and atropine on pepsinogen secretion in the conscious rat

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Summary. Activity of peptic cells is influenced directly by cholinolytic or cholinergic agents. Histamine H_2-antagonists influence the activity of the chief cells through changes of acidity of gastric juice.

The histamine H_2-antagonists inhibit not only the histamine-induced but also the cholinergic-stimulated gastric acid secretion[2-4]. Whether the H_2-antagonists are also able to influence the pepsinogen secretion remains to be clarified. Konturek et al.[5] found that pepsinogen secretion was inhibited by metiamide, but this effect of metiamide was not shown in the experiments of Gibson et al.[6]. They showed that while metiamide inhibited the pepsinogen secretion stimulated by pentagastrin, the cholinergic-induced pepsinogen secretion could not be diminished and was in fact enhanced.

In our experiments, we wanted to investigate the role of histamine H_2-receptors and cholinergic receptors in pepsinogen secretion.

Method. Chronic gastric fistulae were prepared in pentobarbitone (30 kg mg^{-1} i.p.) anaesthetized male albino rats (SIV 50, Ivanovas, Kisslegg) weighing 200–230 g according to Lane et al.[7]. A postoperative period of 2 weeks was allowed. Before each experiment, the rats were starved for 48 h and kept in individual cages to prevent coprophagia. The collection of gastric juice was carried out in modified Bollman-cages. The stomachs were washed with warm water (ca. 50 ml). The 1st fraction (60 min) of gastric juice was eliminated because the distension of the stomach wall by the wash can falsify the secretory values. After this the gastric secretion was collected each hour. The gastric juice was centrifuged, the volume measured, and the acid content titrated by an autoburette (ABU 12, Radiometer, Copenhagen, Denmark). The pepsin activity was determined by the method of Debnath et al.[8] using hemoglobin as substrate and expressed in μmoles splitted tyrosine/min.

The gastric secretion was stimulated by histamine (as hydrochloride, Fluka AG, Buchs, Switzerland), pentagastrin (Gastrodiagnost, Merck AG, Darmstadt) and carbachol (Doryl, Merck AG, Darmstadt) infused into the tail vein in stepwise increasing dosis. Metiamide (Smith, Kline and French Labs Ltd, Welwyn Garden City,