Neurohumoral Correlates of Sleep: Increase of Proteins During Rapid Eye Movement Sleep[1]

The neurohumoral basis of sleep has been a topic of interest particularly during the last 15 years. During this time, special interest has been given to the biogenic amines. However, other substances of a peptidic nature have been reported to be importantly related to sleep[2-4]. In recent years, we have demonstrated that a perfusate extracted, by way of a 'push-pull' cannula, from the midbrain reticular formation (MRF) of sleeping cats, can induce sleep when re-perfused into the homologous MRF of an awake recipient cat[5,6]. We have further shown, that these perfusates contain large amounts of proteins, and that, during a 24-hour perfusion-EEG recording session, the protein levels vary in a cyclic fashion and the peaks of protein levels have a tendency to correlate with periods in which rapid eye movement (REM) sleep occupies a large period of time[7,8]. In view of the suggestion that REM sleep may be accompanied by increases in proteins, this study was designed to investigate whether protein levels in perfusates varied when comparisons were made between wakefulness and REM sleep only.

Methods and results. 14 cats weighing between 2.5 and 3.5 kg were used in this study. Under nembutal anesthesia, cats were stereotaxically implanted with a push-pull cannula system in the reticular formation[6]. In addition, they were supplied with electrodes for recording cortical EEG, eye movements (EM) and electromyogram (EMG) of neck muscles. The electrodes were soldered to a miniature connector and the whole assembly was fixed to the skull with dental acrylic. After a minimum of 10 days of post-operative recovery, the animals were introduced into a cage in a quiet laboratory room, and connected to a Model 7 Grass polygraph. In addition, the push-pull cannula was connected through polyethylene tubes to a Harvard infusion-withdrawal pump. The push side is filled with Ringer solution[6]. When the cat is awake (as determined by EEG recordings), the perfusion is started, with the pump working at a flow rate of 20 µl/min. Thus a 1 ml sample is collected while the cat is awake. As soon as this sample is collected, the animal is allowed to sleep. At this point, perfusions are carried out only while the animal is in REM sleep. In these cases, perfusions are done in as many REM periods as will permit the collection of 1 ml. As during wakefulness, REM is determined behaviourally and electrophysiologically. Each sample is then analyzed independently by the LOWRY[9] method in order to determine total protein content in the perfusate.

As can be seen from the Table, in 21 out of 23 cases protein levels in the perfusates were higher during REM sleep than during wakefulness. A paired *t*-test between the mean awake and mean REM proteins showed a highly significant difference ($p < 0.0001$). It should be noted that protein levels varied between cats as well as within the same cat perfused on different occasions. Despite these variations, however, the awake-REM ratio was always practically double if not more.

Conclusion. These experiments demonstrate that the REM phase of sleep is accompanied by an increase in the levels of extracellular proteins in the midbrain reticular formation. Even though it is not possible from the results to determine the processes whereby these proteins acquire a higher level during REM sleep, it could be suggested that increased protein synthesis may be involved. This suggestion is supported by experiments which have shown that the amount of soluble proteins, in an area 4 to 5 times greater than that occupied by the push-pull cannula, is some 15 times smaller[7]. Moreover, it has been shown that situations which show high levels of protein synthesis, such as in alcohol abstinence[10], neonatal period[11] and during recovery from cycloheximide treatment[12], are accompanied by elevated REM periods[12-14]. Furthermore, it has recently been shown that anisomycin, a protein synthesis inhibitor blocks REM sleep in rats[15]

Protein levels in the perfusates during REM Sleep and wakefulness
µg Protein/ml perfusate

Cat No.	Awake	REM
1	76	133
1	40	128
1	12	43
1	58	23
2	51	197
2	35	162
3	36	96
6	98	262
7	143	170
7	11	46
9	53	166
10	127	123
12	106	232
12	123	176
13	48	253
13	83	140
14	88	130
23	7	34
24	29	46
24	19	53
27	5	15
28	7	34
Mean	56.34	124.91
S.E.	8.46	14.87

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while growth hormone (an anabolic hormone) increases REM sleep in rats[15] and cats[16]. In sum, our experiments suggest that proteins may be importantly related to sleep and in particular to REM.

Résumé. Des chats auquels on a implanté stereotaxiquement un système de cannules «push-pull» dans la formation reticulée mésencephalique ont été soumis à des périodes de perfusion pendant la veille ou pendant la phase REM du sommeil. Les expériences ont démontré que la phase REM est accompagnée d'une augmentation très significative ($p < 0.0001$) des proteines en comparaison avec la veille. Ces résultats sont discutés par rapport au rôle possible de la synthèse des protéines pendant la phase de sommeil.

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Potentiating Effect of β-Adrenergic Stimulant on the Response of the Cutaneous Vascular Resistance to α-Adrenergic Stimulant

It is well known that a stimulation of adrenergic β-receptors causes the relaxation of vascular smooth muscles, particularly in the skeletal muscle and coronary arteries which have relatively increased myogenic tone. On the other hand, the myogenic tone of the cutaneous resistance vessels is relatively decreased and a stimulation of their adrenergic β-receptors does not necessarily produce the relaxation. Furthermore, the cutaneous artery is one of the arteries which have the most sensitive response to vasoconstrictor agents in the body. Recently, many investigators have reported that the β-receptor adrenergic blocking agents have hypotensive action in hypertensive patients[1–3]. It is, therefore, of interest to elucidate the pharmacological characteristics of the adrenergic β-receptors in the resistance vessels.

The purpose of this paper is to evaluate an effect of β-adrenergic stimulation on the response to catecholamines (CA) using the isolated rabbit ear, which consists almost entirely of the cutaneous vascular beds. Male rabbits weighing 2.0 to 3.0 kg were anesthetized with sodium isomital (1.0 mg/kg i.v.) following heparin injection (1000 U/kg i.v.). The central arteries of bilateral ears were cannulated with fine polyethylene tubing and about 1 cm of cannula was inserted into them, then the ears were removed at their bases. They were stored at 3–4 °C in normal Krebs bicarbonate solution for 24 to 48 h. Under these conditions, the degeneration of sympathetic nerves had occurred and their function had failed[4]. The central arteries were perfused with normal Krebs bicarbonate solution, equilibrated with a gas mixture of 95% O_2 + 5% CO_2, at 37 °C by means of a roller pump delivering a constant rate of flow (1.25 ml/min). This solution had the following composition, in mM: Na^+ 162.0, K^+ 5.9, Ca^{++} 2.5, Mg^{++} 1.25, Cl^- 150.4, HCO_3^- 25.0 and glucose 8.3 (pH 7.4). Prior to each experiment, perfusion was always made for 2 h in order to obtain a constant condition. Changes in the vascular resistance were recorded on the kymograph as changes in perfusion pressure with a mercury manometer. 0.1 ml of suitable concentrations of CA, freshly prepared in normal Krebs solution, was injected intraarterially through a rubber tube connected close to the central arterial cannula. To observe an effect of the drugs on the response to CA, the perfusion was performed with normal Krebs solution containing their suitable concentrations for 30 min, and then the responses were compared between those before and during the perfusion.

In the present experiment, the relative potencies of adrenaline (A) (0.1 μg), noradrenaline (NA) (0.1 μg), methoxamine (MX) (0.1 μg) and MX (1.0 μg) were approximately 2.0: 1.0: 0.3: 0.5. In comparison with NA (0.1 μg), the response to A (0.1 μg) was significantly greater ($p < 0.01$), and the responses to 0.1 μg and 1.0 μg of MX were significantly smaller ($p < 0.01$). In the presence of L-isoproterenol (IP) of 10^{-10} to 10^{-6} g/ml, perfusion pressure did not change. As shown in Figure 1, IP (2×10^{-10} g/ml) potentiated the responses to both NA and MX. It was reported that a few min after the stimulation of the adrenergic β-receptors, cyclic adenosine 3′, 5′-monophosphate (cyclic AMP) levels in the vascular smooth

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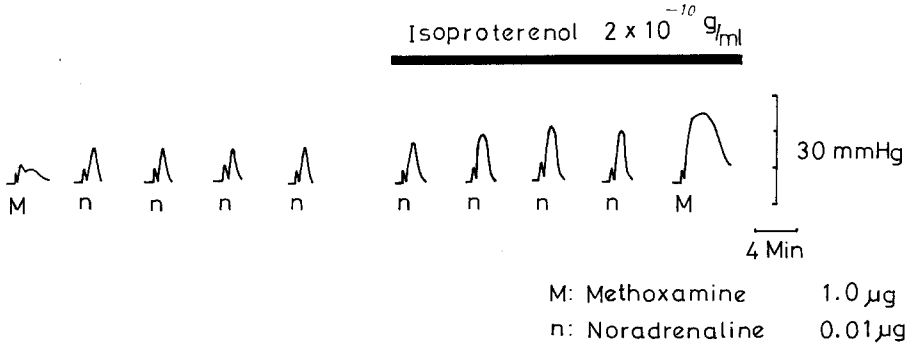

Fig. 1. Effect of L-isoproterenol on the responses of peripheral vascular resistance to catecholamines. Noradrenaline was repeatedly applied every 15 min.