Uptake of Tritiated Glycine into Neurons of the Human Retina

Glycine has recently been shown[1-5] to be taken up by an active mechanism into neurons of the rat spinal cord. Rabbit retina has a similar uptake mechanism[6]. Glycine is now considered a possible neurotransmitter substance in the mammalian central nervous system[6,7], and it has been shown to have inhibitory effects also in the retina[8]. Autoradiographic uptake studies in the rabbit retina suggest that the nerve cells putatively operating with glycine as neurotransmitter are to be sought among the diffuse type amacrine cells[9,10]. Previous work with retinal dopamine-containing ('adrenergic') neurons (see EHINGER and FALCK[11]) has shown that there are considerable differences in the pattern of distribution of adrenergic nerve terminals in different species, even when these are as closely related as different primate species or different rodents. With the autoradiographic method for identifying cells putatively operating with glycine as neurotransmitter available it is of interest to study the uptake of glycine into the human retina in order to establish to what extent the uptake system for glycine in the human CNS resembles that of lower animals and to what extent the uptake is localized to similar cells.

Pieces of retina were obtained from 2 patients (63 and 70 years old) operated on for malignant melanoma of the chorioid. The tissue pieces were taken immediately after the enucleation of the eye, care being taken to avoid the tumour site. The tissue was kept in a solution according to AMES[12] in one case, and ordinary Krebs-Ringer solution in the other. The temperature was about 10–15°C during the transportation to the laboratory (15 min) where they were put into the same solution at 37°C for 10 min. Tritiated glycine (The Radiochemical Centre, Amersham, 2C/mM glycine-2-H 3) was then added to a final concentration of 0.9×10^{-7} M and the incubation was continued for 15 min at 37°C. The incubation solution was kept well aerated with a gas mixture containing 95% O_2 and 5% CO_2. The tissue pieces were subsequently rinsed for 20 min at +4°C in 2 changes of the incubation solution without glycine, frozen in a propane-propylene mixture cooled by liquid nitrogen, freeze-dried, infiltrated with plastic (Durcopan ACM), sectioned on an LKB

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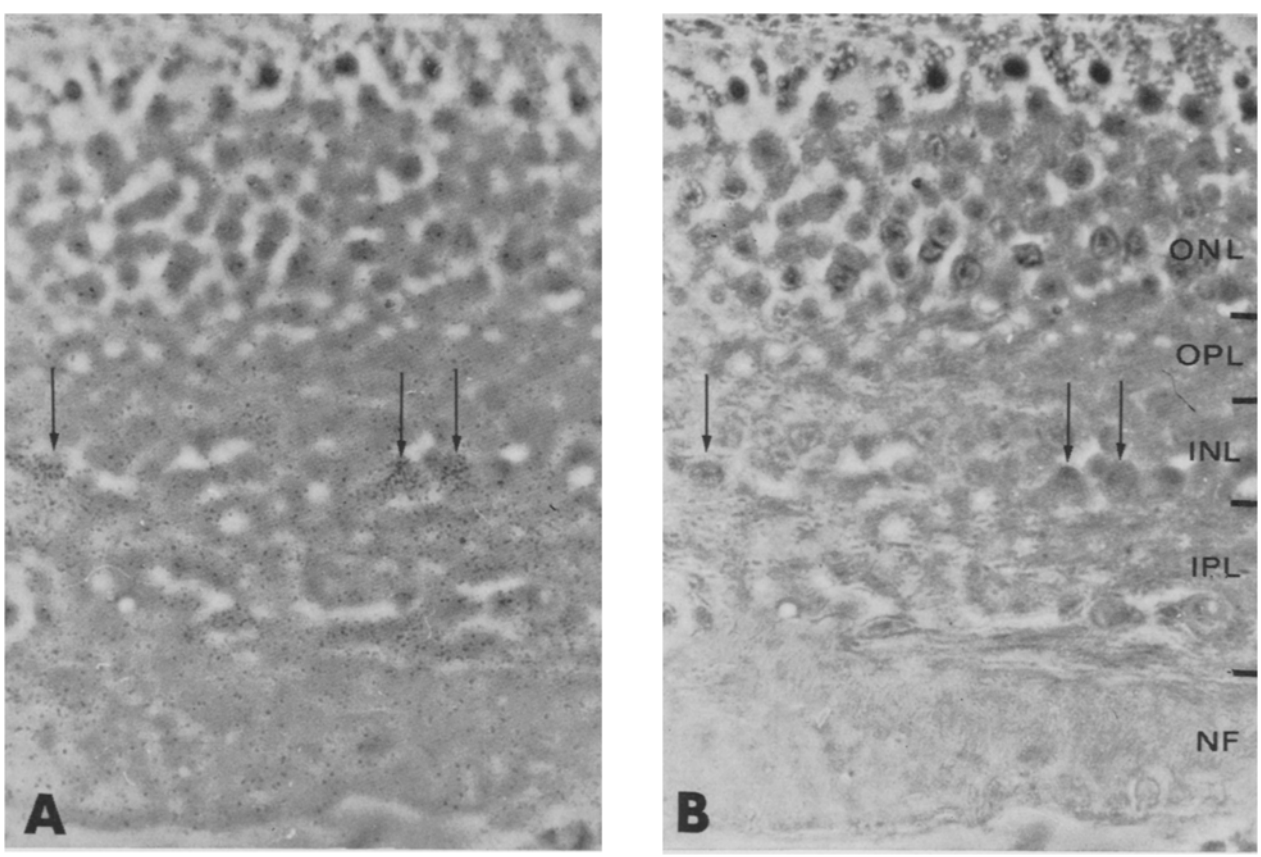

Human retina, incubated in Krebs-Ringer solution with H3-glycine ($0.9 \times 10^{-7} M$) 15 min at 37°C. A) focus on the photographic silver grains. B) same area as A, but with focus on the tissue section. There is radioactivity in some of the cells with the position of amacrine cells (arrows), in the inner plexiform layer (IPL) and to some extent in the nerve fibre layer (NF). There is only little radioactivity in the other layers. OPL, outer plexiform layer; ONL, outer nuclear layer. Phase contrast micrographs ×8900.

Pyramitone, and covered with autoradiographic stripping film (Kodak AR 10), as described previously[9]. The exposure time was approximately 3 months.

In the autoradiographs (Figure) radioactivity was observed mainly over the inner plexiform layer of the retina with no apparent sublayering. There was also considerable activity over the nerve fibre layer. Certain cells with the position of amacrine cells (in the innermost cells of the inner nuclear layer) also showed marked radioactivity. The remaining parts of the retina showed only a slight and diffusely distributed radioactivity. Compared with the pattern seen in autoradiographs made from retinas from rabbits, rats, and guinea-pigs[9,13] produced under similar experimental conditions, the similarity is striking. In rat and rabbit central nervous tissue, it is known that the glycine taken up into neurons remains as such to a remarkable extent in short term incubation experiments[2,5,9]. In these animals, it is also known that there is an active uptake system for glycine, both in the spinal cord[5] and in the retina[6]. The striking similarity between the distribution of radioactivity seen in the human retina and the rabbit, rat, and guinea-pig retinas strongly suggests that in the human retina there is also a very effective neuronal uptake system for glycine. As in the lower animals, there are good reasons for believing that neurons putatively being glycinergic are to be sought among the ones actively taking up glycine[6,9]; in the human retina, certain amacrines are thus possibly glycinergic. As far as the retina can be taken as a model of the situation in the whole brain, the experiment suggests that glycine may be a neurotransmitter also in the human central nervous system[14].

Résumé. L'accumulation de ^{3}H-glucine de la rétine humaine a été étudiée autoradiografiquement. Comme chez les animaux inférieurs, la radioactivité s'accumulait surtout dans la couche plexiforme interne, dans la couche des filaments nerveux et dans des cellules ayant la position des amacrines. Les résultats doivent indiquer que la glucine peut être neurotransmetteuse inhibitive dans l'espèce diffuse des cellules amacrines de l'œil humain et également dans d'autres cellules du système nerveux central de l'homme.

B. Ehinger

Departments of Experimental Ophthalmology and Histology, University of Lund, S-22362 Lund (Sweden), 7 March 1972.

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Sleep in Parabiosis

The nature of sleep has been a topic of interest, and recent sleep research has gradually clarified the sleep-inducing mechanism. Factors involved in induction of sleep phenomena are in general classified as neural and humoral. From the study of Matsumoto and Jouvet[1], monoamines in the brain are considered to be the potent humoral factors.

There are reports of other factors – sleep-producing substances – such as Pieron's classical report on 'hypnotoxin', and recent reports on unidentified 'dialyzable sleep-promoting material'[2–4]. However, these materials were demonstrated by unphysiological procedures, such as restricted condition, crossed-circulation, puncture, injection etc, and recently Ringle and Herndon[5] failed to obtain these sleep-inducing dialysates from sleep-deprived rabbits.

Alekseeva[6] studied pairs of Siamese twins under physiological conditions, and could find no evidence for involvement of humoral factors in sleep mechanisms, since one twin slept while the other was awake. Due to the status of knowledge on sleep at the time of her work, she only examined slow-wave sleep (SS), which is greately affected by neural factors, including behavior and higher nervous activity, and did not examine paradoxical sleep (PS).

The present study was to see whether SS and PS appeared synchronously in parabiotic rats. It is reasonable to presume that sleep would become more synchronized in proportion to the degree of homeostasis between the parabiotic rats.

Method. Male, Wistar strain rats, weighing 150 to 250 g, from different litters were connected parabiotically by a modification of the Bunster and Meyer[7] method under Nembutal narcosis. In the early stage of experiments a parabiotic union was performed 7 to 10 days after operation for polygraphic recording on a single rat, but later the order of the procedure was reversed. Electrodes were implanted on each parabiotic rat under ether anesthesia as described in our previous report[8]. 70 pairs were operated twice, of which 32 pairs died within 3 days after the last operation. The synchronization of sleep in 38 healthy parabiotic pairs with chronically implanted electrodes were compared with those in 25 control pairs united by their skins only.

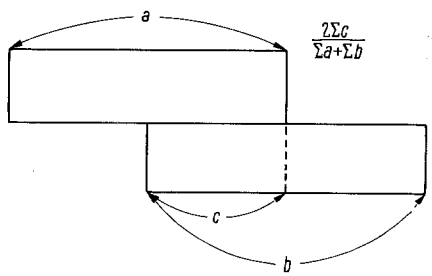

Fig. 1. Interpretation for the synchronization rate.

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