volume of 0.5 ml: 60 mM tris-HCl (pH 7.4), 1 mM dithiothreitol, 50 mM KCl, 4 mM $MgCl_2$, 0.3 mM GTP, 1 mM ATP, 5 mM phosphoenolpyruvate, 10 μg pyruvate kinase, pH 5 enzyme (600 μg protein), amino acyl transferases [200 μg protein of 35–65% $(NH_4)_2$ SO_4 fraction], 2 μmoles of ^{14}C-L-leucine (sp. act. 280 mCi/mmole, New England Nucl. Corp.) and 4 mM of each of 19 L-amino acids except leucine. The reaction was started by ribosomes ($10A_{260\,nm}$ units) and run for 15 min at 37 °C (linear for this time). Polypeptide synthesized was measured by the radioactivity incorporated into trichloracetic acid-insol. material as described before[8]. Fractions of microsomal extracts, tested in the above system, were obtained as follows: Microsomes were sonicated (M.S.E. sonicator, 2 amp. current output, 1 min at 0 °C) in a medium (microsomes equivalent to 1 g liver/0.6 ml of 5 times diluted buffer, pH 7.4 containing 20 mM tris-HCl, 1 mM dithiothreitol, 50 mM KCl and 4 mM $MgCl_2$). An aliquot (20 mg protein) of the clear sonicate (obtained by centrifuging at 105,000 × g for 90 min at 4 °C) was than chromatographed on a sephadex G-200 (Pharmacia) gel column (20 × 1.8 cm) and elution of fractions being done by the same buffer. 40 drops were collected per fraction and 0.2 ml of suitable fractions tested for their effects in the cell-free system. The ages of the 'young' and 'aged' rats were 3 weeks and 1 year respectively.

Results and discussion. Figure 1 compares between young and aged rats the rates of ^{14}C-leucine incorporation by the liver microsomes as well as ribosomes. Each point gives the ratio of the values for radioactivity incorporated by the sample from the young rat to that by the sample from the aged animal; a value greater than 1 signifies an activity higher for the test material from the young as compared to that from the aged. It is seen that at all concentrations tested, the young microsomes have a higher capacity for polypeptide synthesis than the aged microsomes, the difference being specially marked at the lower concentrations. The ribosomal activities in the 2 age-groups are, however, comparable. Figure 2 shows the effects of fractions from the extracts of microsomes of young and aged rats, on cell-free polypeptide synthesis. It is observed that the microsomes of both young and aged rats contain an activator as well as an inhibitor. But in the young, the level of the activator is conspicuously higher and that of the inhibitor lower than the corresponding levels in the aged.

The nature of the 2 'factors' is yet to be clarified. But a role in physiological regulation is expected, in view of the fact that these are associated with microsomes which are the sites of cellular protein synthesis. We[3] found the specific activity (per 100 μg protein) of the activator to that of the inhibitor to be 5–10 in the young microsomes as against only 1 in the aged. The higher ratio in the young is in keeping with their higher capacity to synthesize protein.

Hoagland et al.[9] found in liver microsomes a heat-labile inhibitor whose effect was antagonized by GTP. The inhibitor, described here, is heat-stable (90 °C for 5 min) and its activity, in our hands, is not counteracted even by a 5fold increase of GTP. The inhibitor was tested in this study by using the rat liver system requiring 4 mM Mg^{+2}. It has since been revealed[10] that the same inhibitor is twice as effective in inhibiting ^{14}C-phenylalanine incorporation in a polyuridylic acid (poly U)-dependent polyphenylalanine synthesizing system with 10 mM Mg^{+2}. Using this system, we found[11] that the site of action of the inhibitor lies in the step leading to aminoacylation of tRNA, a reaction prerequisite for protein synthesis. The activator, on the other hand, was found to be heat-labile and lost 50–60% of its stimulatory activity in 72 h even when kept frozen at −20 °C[12]. It showed maximum effect in the rat liver system at 4 mM Mg^{+2}, being totally ineffective in the poly U-directed polyphenylalanine synthesizing system under optimal condition (10 mM Mg^{+2}). Interestingly, these characteristics are reminiscent of those ascribed originally by Miller and Schweet[13] to reticulocyte ribosomal extract known to contain protein initiation factors. Work is underway to delineate the site of action of the activator. This is of particular interest, since the same activator appears also to be implicated in changes associated with other physiological conditions, such as hormone action. Thus, administration of glucocorticoids to adrenalectomized rats induced an increase in the level of the activator in the liver microsomes and concomittantly led to an increased ability for protein synthesis by those microsomes[12].

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Light evoked release of radioactivity from rabbit retinas preloaded with (^{3}H)-GABA[1]

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Summary. Light flashes evoke an increased release of radioactivity in vitro from rabbit retinas preloaded with (^{3}H)-GABA in vivo. Constant light does not affect the release. No light evoked release can be demonstrated from the glia. Pentobarbitone and AOAA depress the evoked release. The results are consistent with GABA being a retinal neurotransmitter, most likely in a class of amacrines.

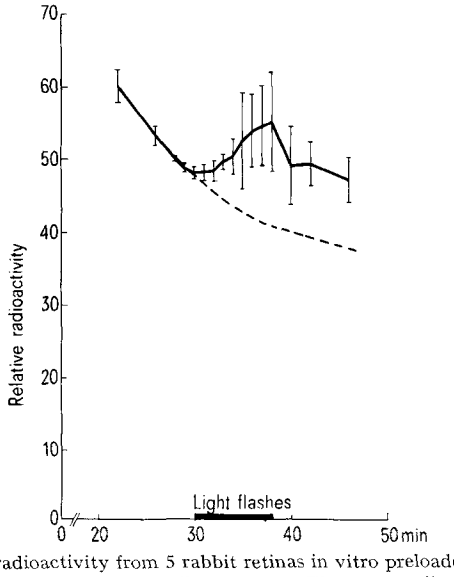
There is now increasing evidence that γ-aminobutyric acid (GABA) functions as a neurotransmitter in the vertebrate retinas. It occurs naturally in retinal tissue[2–4] and a high affinity uptake system[5–7] and enzyme systems for its formation and metabolism have also been demonstrated[4, 8–10]. In rabbit retina, exogenous GABA is taken up in vivo in cells that have, in the main, the location and distribution of amacrine cells, but also in some ganglion cells[11, 12]. Moreover GABA has an inhibitory effect on the firing of retinal ganglion cells[13–16], and the GABA inhibitors picrotoxin, bicuculline and N-methyl bicuculline affect retinal function[14, 17, 18].

Electrical stimulation and high potassium concentration increase the spontaneous release of labelled GABA from the retina[19]. However, both these stimulation methods are crude and can release GABA from tissue where it is unlikely to be a neurotransmitter[20]. The retina offers the advantage of responding to light stimulation also when isolated in vitro. We have therefore studied the possibilities of releasing GABA from rabbit retina in vitro by flashing light stimulation.

(^{3}H)-GABA was injected intravitreally into the eye with topical anaesthesia only. 2 or 4 h after the injection the rabbit was killed by air i.v. and the eye enucleated. The anterior segment and vitreous were carefully removed. The eye cup was turned inside out and placed in a water-jacketed superfusion chamber specially designed to minimize dilution effects. This, together with the choice of species, may in part explain why in previous studies a light-evoked release of labelled GABA was not seen[19,21]. The retina was superfused at a rate of 1 ml/min with the solution described by Ames[22]. 1 mM unlabelled GABA was added and, in some experiments, 0.1 mM AOAA was also present. The solution was gassed with a 95% O_2 and 5% CO_2 gas mixture and was maintained at 37 °C. After 30 min superfusion in the dark, the retina was stimulated by light flashes for a period of 8 min at 2 flashes/sec from a xenon flash tube. The average illumination on the retina was 1.75 lux, but the peak of the flash began with an illumination of 2175 lux and decreased exponentially with a time constant of 0.4 msec.

The radioactivity of the superfusate was monitored by liquid scintillation spectrometry. The site of uptake of radioactivity in retina was studied by autoradiography and, 4 h after the injection, was found mainly in cells with the location of amacrines, in good agreement with previous results[12], and 2 h after the injection mainly in glia. There was also radioactivity in nerve cells but these were largely disguised by the glial radioactivity.

The actual level of radioactivity in the superfusate varied between different experiments, no doubt because of variations in the initial in vivo labelling of the retina. Therefore the efflux curves were computer fitted to each other so that the 5 min preceding the light stimulation would attain a fixed position on the plot.



Efflux of radioactivity from 5 rabbit retinas in vitro preloaded with (^{3}H)-GABA stimulated with light flashes. The broken line is the spontaneous efflux of radioactivity when retinas are kept in the dark. The deviation caused by the light stimulation is highly significant ($p < 0.005$). SE of mean are indicated by vertical bars.

Stimulation of the 4 h preloaded retinas with light flashes increased the release of radioactivity significantly (figure) ($p < 0.005$ when the slope was compared with the slope of the release curve in the dark by t-test on linear regression for the interval 30–38 min). From uptake studies in both rabbits[23] and other species[5,6,24], it is known that most of the stored radioactivity represents unchanged GABA. Any radioactivity released is thus likely to originate from stored ^{3}H-GABA, but since GABA is fairly rapidly metabolized, it is likely that most of the radioactivity in the superfusate consisted of metabolites of GABA[5,19,25]. When the metabolism of GABA was inhibited by 0.1 mM AOAA in the superfusion medium, there was on the average a decrease in the level of efflux of radioactivity from the retina as has been noted previously[5,19,25], but flashing light still increased the efflux of radioactivity slightly, albeit not significantly. Anaesthetizing the animal with pentobarbitone instead of killing it with air i.v. blocked the release of radioactivity. Pentobarbitone has been demonstrated to inhibit GABA uptake and reduce GABA efflux[26].

If the superfusion was started 2 h after the intravitreal injection of ^{3}H-GABA when most of the radioactivity in the retina was found in glia, no light-induced release of radioactivity was observed. A small neuronal release in these experiments is likely to be concealed by the dominating spontaneous glial efflux. Since neuronally evoked release of a substance is one of the main criteria for a neurotransmitter[27], our present results offer evidence compatible with GABA being a retinal neurotransmitter. It is important that the release can be achieved with a physiological stimulus, as it suggests that it is the result of normal nerve activity.

1 This work was supported by grants from the Swedish Medical Research Council (project 04X2321), The Faculty of Medicine, University of Lund, Royal Physiographic Society.

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