urethane anaesthetized rats, desheathed, and the acetylcholine stores labelled with [^{3}H]-choline (1 μM; 13 Ci/mmol) as described by Higgins & Neal (1978). The ganglion was then mounted in a three chambered bath like that described by Bowery & Tulett (1975) and superfused at 27°C with oxygenated Krebs containing neostigmine (50 μM) at 0.5 ml/min. After 60 min perfusion the ganglion nerve trunk was stimulated for 2 min every 16 min (supramaximal voltage; 0.5 ms). Effluent radioactivity was collected at 4 min intervals and measured. Ganglionic action potentials were monitored with reference to the post-ganglionic trunk; presynaptic d.c. potential changes were measured between the ganglion and the preganglionic nerve trunk.

Application of GABA (10–1000 μM) to the ganglion (2 min, commencing 30 sec before nerve stimulation) reduced evoked tritium release by up to 30% (Figure 1). In the absence of nerve stimulation, GABA slightly increased basal tritium release. In comparable concentrations, GABA depolarized the presynaptic elements in the ganglion. The relationship between presynaptic depolarization and inhibition of release is under further investigation.

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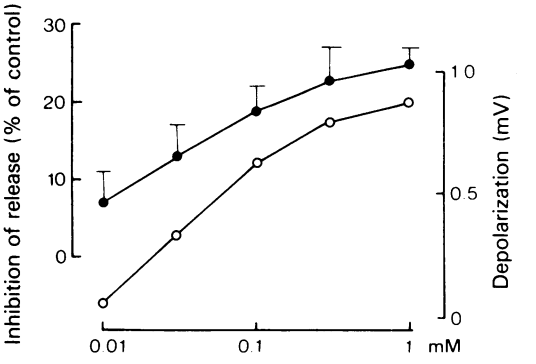

Figure 1. Inhibition of (●) evoked tritium release and (○) presynaptic depolarization produced by GABA in isolated rat superior cervical ganglia. ●, means (+ s.e.) of 3 release experiments; ○, single (separate) experiment.

Amino acid transmitter candidates of the rat olfactory cortex

G.G.S. COLLINS

Department of Pharmacology and Therapeutics, University of Sheffield, Sheffield S10 2TN

The isolated perfused rat olfactory cortex slice provides a useful preparation for the study of central synaptic mechanisms (Pickles & Simmonds, 1976). The preparation has now been used in an attempt to identify which amino acids, if any, are neurotransmitter substances in this brain area.

Slices of rat olfactory cortex were prepared, incubated and perfused (25°C) as described previously (Pickles & Simmonds, 1976). A perspex cylinder, containing 50 μl of the perfusion medium, was placed on the uncut surface of the slice and the solution changed every 10 min. The aspartate, glutamate, GABA, glycine, taurine, alanine and glutamine content of the solution was estimated using a sensitive double-isotope dansylation procedure (Clark & Collins, 1976). On supramaximal electrical stimulation of the excitatory input to the preparation, the lateral olfactory tract (LOT), at a rate of 4/min for 20 min, there was a significantly ($P<0.05$) increased release of aspartate (212 ± 28 pmole released in excess of a resting efflux of 43 ± 5 pmole/10 min), GABA (137 ± 16 pmole released in excess of a resting efflux of 15 ± 1 pmole/10 min) and taurine (1166 ± 152 pmole released in excess of a resting efflux of 235 ± 15 pmole/10 min) (all values are mean ± s.e. mean; n between 6 and 36). The release of none of the other amino acids was altered significantly. The evoked release of aspartate and GABA, but not that of taurine, was completely abolished in the absence of Ca^{++}.

The studies suggest that aspartate and GABA may be neurotransmitters in this brain area and in order to investigate this possibility, two further series of experiments were performed;

1. When the depth distribution of the amino acids was determined, the GABA concentration was relatively low to a depth of 200 μm but then progressively increased to a depth of at least 500 μm. In contrast, the concentrations of both glutamate and aspartate were relatively constant between 200 and 500 μm. The distribution of taurine, glycine and alanine was uniform throughout.

2. When groups of rats were chronically, unilaterally bulbectomized, there was a specific, significant reduction in the tissue content of aspartate 2 days following surgery (from 2.92 ± 0.37 to 1.51 ± 0.17 μmole/g; $n = 6$) which was accompanied by a failure of LOT stimulation to release any of the amino acids.

These results differ from those of Bradford & Richards (1976) and of Yamamoto & Matsui (1976) who found that electrical stimulation of the LOT of guinea-pig olfactory cortex slices evoked a specific release of glutamate. This discrepancy could be the result of differences in stimulation frequency, perfusion temperature, incubation procedure and animal species used. Nevertheless, the present results suggest that some of the LOT fibres utilized aspartate as an exitatory transmitter whereas GABA is an inhibitory transmitter released from deeper lying fibre terminals.

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The release of amino acids and [^{3}H]-ACh from the rabbit retina *in vivo*

G.G.S. COLLINS, S.C. MASSEY & M.J. NEAL

Department of Pharmacology, The School of Pharmacy, 29/39 Brunswick Square, London WC1N 1AX

When photoreceptors in the vertebrate retina are stimulated with light, horizontal cells and some bipolar cells respond by hyperpolarizing. These responses are believed to be due to a reduction in the release of a depolarizing transmitter from the photoreceptor terminals, and it has been suggested on electrophysiological evidence that aspartate may be this photoreceptor transmitter substance (Wu & Dowling, 1978).

In the present experiments, we have attempted to obtain further information on the photoreceptor transmitter by studying the effect of photic stimulation on the release of amino acids and [^{3}H]-ACh from the rabbit retina *in vivo*.

Rabbits were anaesthetized with urethane and in each experiment one eye was sutured to a ring for support. The cornea, iris, lens and vitreous were then removed and the resulting 'eye-cup' was filled with Krebs bicarbonate Ringer containing [^{3}H]-choline (10 μM, 13 Ci/mmol). After 30 min, the retina was irrigated with fresh medium containing eserine (30 μM) for 60 min and then 0.4 ml of medium containing eserine was placed in the eye-cup. This medium was replaced at 10 min intervals and the [^{3}H]-ACh in the resulting samples was determined as described previously (Massey & Neal, 1978). The amino-acids in the samples were measured using a radiochemical dansyl derivative technique (Clark & Collins, 1976). The retina was stimulated with flashes of light from a Devices photic stimulator and the physiological response of the retina was assessed during the experiments by recording the electro-retinogram (erg).

Stimulation of the dark-adapted retina for 10 min with flashes of light (3 Hz, average retinal illuminance 7.6 lux) reduced the release of aspartate from a spontaneous resting release of 143 ± 32.1 to 51.4 ± 13.6 p-mole/10 min (mean ± s.e. mean of 5 experiments, $P<0.05$). In contrast, the release of taurine was increased more than four fold, the resting release of 293 ± 51.9 being increased to 1321 ± 436 p-mole/10 min (mean ± s.e. mean of 5 experiments, $P<0.01$). The release of GABA, glutamate, alanine, glutamine and glycine were unaltered by photic stimulation.

In the same experiments, the release of [^{3}H]-ACh from the retina was increased to 4.1 ± 0.29 ($P<0.01$) times the spontaneous resting release by flashes of light. This light evoked release of [^{3}H]-ACh had previously been shown to be calcium dependent and to be maximal at a stimulus frequency of 3 Hz (Massey & Neal, 1978).

These experiments support the suggestion that aspartate may be the photoreceptor transmitter, since it alone showed a reduced efflux in response to light flashes. Conversely, the increase in release of ACh and taurine in response to photic stimulation suggest their role in the retina is not that of the photoreceptor transmitter substance.

S.C.M. is an SRC student.

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