Uptake of ^{14}C-L-glutamate by rat retina

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For studying synaptic mechanisms in the central nervous system, the retina would appear to have many advantages. It is easily accessible and, unlike brain slices, it can be maintained *in vitro* in a histologically normal and functional condition for many hours. L-Glutamate may be an excitatory synaptic transmitter substance in the central nervous system (Krnjević, 1970) and in this study, the uptake of ^{14}C-L-glutamate by the retina has been investigated. The uptake of putative amino-acid transmitter substances in the brain is of interest as it has been suggested that, after release from nerve terminals, their actions may be terminated by uptake processes, analogous to that for noradrenaline (Iversen & Neal, 1968; Curtis, Duggan & Johnston, 1970; Neal, 1971).

Rats were killed by cervical fracture and the eyes were enucleated. The retinae were rapidly dissected and each was placed in 10 ml of oxygenated Krebs-bicarbonate Ringer. After a preliminary incubation of 15 min at 35°C, ^{14}C-L-glutamic acid ($1{\cdot}2 \times 10^{-8}$ M) was added and the incubation was continued for various times. The retinae were recovered by filtration and after washing with 5 ml of ice-cold medium, the radioactivity in the tissue was determined by liquid scintillation counting.

The retinae rapidly accumulated radioactivity, resulting in a tissue to medium ratio of 45 to 1 after incubation for 60 min at 35°C. Ion-exchange and paper chromatographic analyses of tissue extracts showed that ^{14}C-L-glutamate was considerably metabolized by the retina. Thus, after an incubation of 10 min at 35°C, only about half (54%) of the radioactivity in the tissue was unchanged ^{14}C-L-glutamate. The major radioactive metabolites were glutamine (28%), aspartate (14%) and γ-aminobutyrate (4%). These metabolites were not detected in the medium, indicating that the products of ^{14}C-L-glutamate metabolism were retained by the tissue. The uptake of total radioactivity by the tissue was, therefore, used as a measure of glutamate uptake.

The uptake of ^{14}C-glutamate was temperature dependent, being considerably reduced at 0°C and optimal at 25°C. The uptake of ^{14}C-glutamate exhibited saturation kinetics over a range of external L-glutamate concentrations from 10^{-6} M to 10^{-4} M, with an apparent *Km* for L-glutamate = 16·5 μM and Vmax = (0·03 μmoles/g)/minute. Replacement of sodium chloride in the incubating medium by choline chloride reduced the uptake of ^{14}C-glutamate to less than 5% of control values. A large reduction in uptake also occurred when the tissue was incubated with ouabain (10^{-4} M), dinitrophenol (10^{-3} M) or with *p*-hydroxymercuribenzoate (10^{-5} M). The uptake of ^{14}C-L-glutamate ($1{\cdot}2 \times 10^{-8}$ M) was not affected by the presence of glycine, D,L-homocysteate, L-histidine, L-serine, 2,4-L-diaminobutyrate or γ-aminobutyrate (10^{-3} M) but was significantly reduced by L-aspartate (10^{-3} M).

The results show the existence in the retina of a highly efficient and specific uptake mechanism for L-glutamate. The process shows many of the characteristics of an active transport system.

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Supersensitivity of central noradrenaline receptors after reserpine

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In a recent series of experiments the effects of (−)-noradrenaline (NA), applied by microiontophoresis, on the activity of spontaneously firing cells in the brain stem of rats anaesthetized with halothane have been studied in untreated animals and in animals pretreated with reserpine, α-methyl-*p*-tyrosine (AMPT) or bis(1-methyl-4-homopiperazinylthiocarbonyl)-disulphide (FLA 63). The doses of these drugs and the pretreatment times are given in Table 1. The communication presents data concerning the firing rates of the brain stem neurones in each group and their responses to iontophoretically applied NA.

TABLE 1. *Effects of monoamine depletion by pretreatment with different drugs on neuronal firing rates and neuronal sensitivities to noradrenaline (NA) in the brain stem*

	Neuronal firing rates			Magnitudes of NA excitations	
Drug, dose and pretreatment time	Median (spikes s^{-1})	Mode	(No. of neurones studied)	Mean no. of spikes elicited by 0·75 μC NA	(Sample size)
None	14·9	8·0	(113)	2322	(12)
Reserpine (5 mg/kg) 20 h pretreatment	11·7	2·0	(113)	4242†	(13)
α-Methyl-*p*-tyrosine base (500 mg/kg) 20 h pretreatment	14·4	8·0	(113)	3455‡	(15)
FLA 63 (25 mg/kg) 4 h pretreatment	14·4	12·0	(56)	2270‡	(14)

†Significantly different from untreated values ($P<0·001$). ‡Not significant (Student's *t* test).

The neurones in the untreated animals had median firing rate of 14·9 spikes s^{-1}, and a mode firing rate of eight spikes s^{-1}. The firing rates of the neurones in the animals pretreated with AMPT and FLA 63 were similarly distributed (Table 1). The firing rates of the neurones in the animals pretreated with reserpine were significantly differently distributed, with lower median and mode frequencies (Table 1).

The responses of the brain stem neurones in the rats anaesthetized with halothane resembled those observed in the unanaesthetized decerebrate cat (Boakes, Bradley, Brookes, Candy & Wolstencroft, 1971). The responses to NA in the animals pretreated with AMPT and FLA 63 were similar to those in the untreated animals. In the reserpinized animals the excitatory responses to NA were much greater than those in the other groups, both in magnitude and duration. Table 1 shows an analysis of the responses to NA of a sample of neurones in each group. Few inhibitory responses were observed in the reserpinized animals but these were similar to those observed in the other groups.

The increased sensitivity to NA in the reserpinized animals is similar to the supersensitivity of peripheral structures to NA after reserpine pretreatment (Trendelenburg, 1963). The absence of any change in the responses of the neurones in animals pretreated with AMPT or FLA 63 suggests that the increased responses to NA observed