The Effect of Iontophoretically Applied Acetylcholine upon the Cat’s Retinal Ganglion Cells*

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Summary. The effect of iontophoretically applied acetylcholine was tested in 278 retinal ganglion cells of the cat.

26% of 122 ganglion cells were influenced by acetylcholine. In animals, which were pretreated with intravenously administered physostigmine, the proportion of acetylcholine sensitive cells rose to 87%. Acetylcholine had a differential effect upon retinal reaction types. Off-center neurons were excited, On-center neurons were inhibited. The time course of the acetylcholine effect resembled that observed in the cerebral cortex. In a considerable proportion of units, the effect used to diminish with repeated application of the drug (desensitisation).

Intravenously applied atropine blocked the excitatory response of Off-center neurons to acetylcholine, but failed to prevent acetylcholine inhibition of On-center neurons. The excitatory response appears thus predominantly mediated by muscarinic receptors. Nicotinic receptors are obviously of little importance in mediating the response to acetylcholine, since acetylcholine sensitivity remained unchanged after intravenous injection of the curariform agent dihydro-β-erythroidine. Present histochemical and histological knowledge together with our results suggest, that the response of retinal ganglion cells to light stimulation of their receptive field periphery is possibly transmitted through cholinergic amacrine cells.

Key words: Acetylcholine — Physostigmine — Microelectrophoresis — Ganglion Cells — Retina.

The considerable amount of histochemical indirect evidence, which suggested a transmitter function of acetylcholine in the mammalian retina [13, 16] has recently gained support from observations, that acetylcholine influenced spontaneous and light driven activity of retinal ganglion cells, when applied intraarterially in the cat [18] or when added to the incubation medium of the rabbit’s isolated retina [1]. However, these methods of application did not permit to specify the site of drug action within the retina. The observed effects might reflect direct drug action and/or drug induced input changes. In search for a possible direct effect of acetylcholine upon retinal ganglion cells, we used presently iontophoresis as a method of application, which is supposed to restrict

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drug action predominantly to the recording site. A brief outline of the
results has been given elsewhere [17].

Methods

Preparation. Cats were prepared under ether anesthesia. The head was fixed in
a head frame, the cornea and lens were removed to enable a direct approach to the
retina through the vitreous body. After careful local anesthesia of pressure points
and wound edges, general anesthesia was discontinued, the animal was immobilized
by flaxedil, and given artificial respiration. Expiratory CO₂ and body temperature
were monitored.

Recording and Drug Application. Under visual guidance the multibarrel electrode
was driven through the vitreous body and inserted into the retina by means of a
micromanipulator. Multibarrel electrodes were prepared using methods previously
described by Herz et al. [11]. Pipettes were boiled in distilled water; then the water
was replaced as far as possible by test solutions. Filled micropipettes were stored
at 4°C and used 24 h later. The microelectrophoretic method of controlling eflux of
substances from drug containing barrels has been described previously in detail [14].
Electrophoretic currents were measured with an accuracy of ± 1 nA. Extracellular
spikes were recorded from single retinal units by means of a barrel containing 4 M
NaCl solution. The other three barrels contained acetylcholine (2 M; pH 4), mono-
sodium-glutamate (3 M; pH 8.4) and 3 M NaCl, respectively. The latter served as a
control for current effects. The neuronal signals were fed into a conventional pre-
amplifier and displayed on the oscilloscope where photographic records were taken
from. Discharge rate was determined by counting spikes of film records. For differenti-
atiation of optic nerve fiber- and ganglion cell potentials the following criteria were
used.

1. Spike form and polarity [3].
2. Intraretinal location of recording site indicated by distance from retinal
surface and by polarity and amplitude of the b-wave of the ERG.
3. Responsiveness to glutamate. Spontaneous activity was recorded during a
background illumination of 50 cd/m². Light responses were evoked by intermittent
diffuse illumination of the retina (duration: 1 sec; intensity: 100 cd/cm²). Light
evoked excitation and transient poststimulatory inhibition were taken as indicators
of On-center properties. Units suppressed by light and activated at light off were
classified as Off-center neurons. Neurons with an excitatory response at light on and
light off were termed On-Off neurons.

Results

From 122 ganglion cells studied, only 32 (26%) were affected by
acetylcholine. The fact, that in the small sample of responsive units
acetylcholine in doses of 40—200 nA predominantly suppressed On-units
and activated Off-units, suggested a differential effect of the substance
upon On- and Off-units. The relatively small proportion of acetylcholine
responsive neurons might have represented the true proportion of acetyl-
choline sensitive units in the retinal ganglion cell population. Suspecting
the subsynaptic membrane of ganglion cell dendrites to be a possible site
of acetylcholine action, we assumed that the substance might not have