THE EFFECT OF CHOLINERGIC AGONISTS AND ANTAGONISTS ON GANGLION CELLS IN THE MUDPUPPY RETINA

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INTRODUCTION

The role of acetylcholine in the retina has recently been reviewed by Neal (1) and Puro (2). Although histochemical and immunocytochemical studies indicate the presence of acetylcholine in the retina in many species, physiological evidence for its action as a neurotransmitter has been obtained in only a few of these, including rabbit (3-8), cat (9-11) and carp (12,13). These studies suggest that acetylcholine is used as a transmitter by certain types of amacrine cells and mediates some of the synaptic input to ganglion cells. Therefore it was of interest to investigate the effects of cholinergic agonists and antagonists on ganglion cells in mudpuppy, where other aspects of the physiology of the inner retina have been extensively studied. The results reported here suggest that mudpuppy ganglion cells have nicotinic acetylcholine receptors and that they receive tonic, depolarizing, cholinergic input in darkness. However, neither transient nor sustained responses to stationary light stimuli appear to be mediated by this input.

METHODS

Intracellular recordings were made from ganglion cells in the eyecup of the mudpuppy (*Necturus maculosus*) using micropipettes of 300-800 MΩ resistance filled with 4 M potassium acetate. The eyecup preparation and the stimulating and recording systems have been described in detail previously (14). The eyecup was superfused with a continuous flow of Ringer solution of the following composition (in mM): NaCl 110, KCl 2.5, $CaCl_2$ 1.8, glucose 11, HEPES buffer 5.0, adjusted to a pH of 7.8. During the recording the superfusate could be switched to a solution which was identical except for the addition of specific test substances. Current-voltage relations were made by passing constant current steps through the recording electrode and measuring the resulting steady-state voltage displacements using an active bridge circuit. Current-voltage relations measured when the electrode was outside the cell were used to correct for electrode rectification, which often occurred with current intensities greater than ±0.05 nA. Responses were stored on magnetic tapes and displayed on a chart recorder, from which the illustrated responses were photographed.

The light stimulus was a 100 μm diameter spot of white light centered in the cell's receptive field. The preparation was dark adapted for more than 30 min before experiments were begun. The range of light intensities used was such that both rod and cone responses were elicited, as judged by recordings from horizontal cells, where the two types of inputs could be identified (15). The unattenuated light intensity at the plane of the retina was $4 \cdot 10^{-2}$ μW$\cdot$cm^{-2}. Stimulus intensities in the figures are expressed as $\log_{10}$ units of attenuation by neutral density filters.

RESULTS

Responses to acetylcholine and carbachol

Figure 1 shows the response of an on-center ganglion cell to 5 mM acetylcholine, which caused a depolarization in darkness and a reduction in the amplitude of the light-evoked responses. The same result was observed in all three classes of ganglion cells (on-center, off-center and on-off), to both acetylcholine and carbachol. The reduction in light response was probably due to shunting since the agonists caused a large increase in conductance. Current-voltage relations from another on-center ganglion cell in the presence and absence of acetylcholine are shown in Fig. 2. In this cell 5 mM acetylcholine caused a depolarization of 14 mV and a decrease in input resistance from 160 to 110 MΩ. Both acetylcholine and carbachol were also effective when transmitter release was blocked with 4 mM cobalt chloride, indicating that this was a direct effect of acetylcholine and carbachol on the ganglion cell rather than an indirect effect via a presynaptic cell. Figure 3A shows the response of an off-center ganglion cell to carbachol in the presence of Co^{2+}.
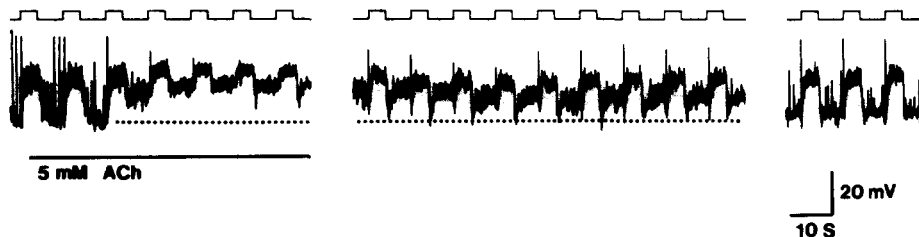

5 mM ACh

20 mV

10 S

Fig. 1. Effect of acetylcholine on an on-center ganglion cell. Solid horizontal line under response trace indicates presence of 5 mM ACh. Dashed horizontal line indicates level of membrane potential in darkness before application of ACh. Gaps in the traces are each 1 min duration. Light stimuli (intensity -4.8, duration 5 sec) indicated by upward deflection of the top trace.
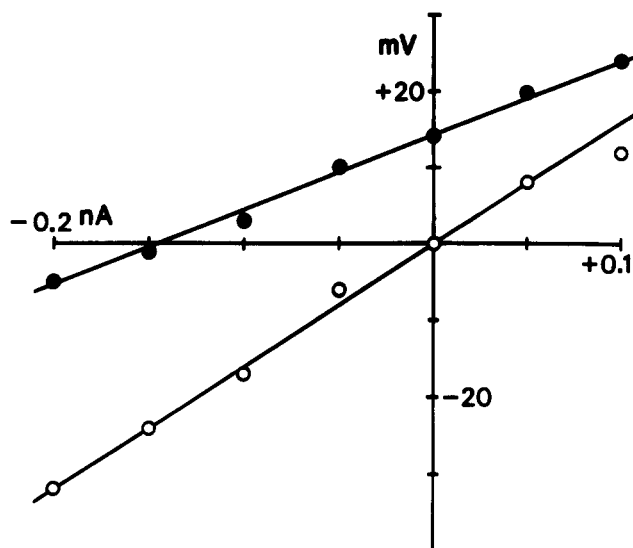
Fig. 2. Current-voltage relations of an on-center ganglion cell in darkness in the absence (o) and presence (●) of 5 mM acetylcholine. Potential changes are plotted relative to control membrane potential in darkness.

The responses of all ganglion cells to carbachol were of long duration. Even with brief exposures, as in Fig. 3A, it often took more than 20 min for the membrane potential to recover to the control value. The recovery of membrane resistance was often more rapid than that of membrane potential. For example, in Fig. 3A the break near the end of the trace indicates a period of 6 min. After this period the membrane potential had changed only slightly, but membrane resistance had nearly doubled. Measurements before and after the recording confirmed that the resistance changes were not due to changes in electrode properties. A possible explanation for the lack of correspondence between membrane potential and resistance during recovery may be that carbachol acts with different time courses at two (or more) types of receptors which control different ionic conductances.

In the cell described in Fig. 3A the bathing solution was switched back to control Ringer before the response to carbachol had reached its peak. In the continued presence of carbachol (Fig. 3B) both the voltage response and the conductance increase reached an initial peak and then declined back to a lower value, suggesting that there was some kind of desensitization of the acetylcholine receptors. The degree of desensitization, as judged by the amount of this decline, was quite variable and did not seem to be related to the type of
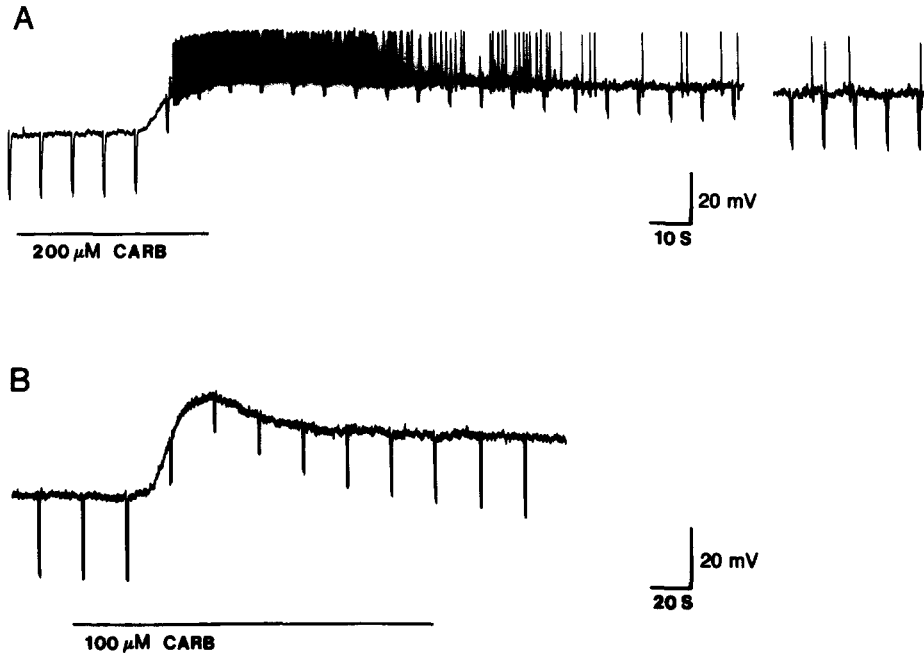
Fig. 3. Effect of carbachol on off-center ganglion cells. A and B are different cells. A, 200 μM carbachol in normal Ringer solution; B, 100 μM carbachol in the presence of 4 mM cobalt chloride. Downward deflections of trace are voltage drops produced by -0.04 nA current pulses. Break in trace A is 6 min duration.

ganglion cell or the concentration of carbachol. A second exposure to carbachol within the next 20 min usually had little or no effect. Because of this it was not possible to determine dose response curves in a given cell. However, based on the responses of 50 ganglion cells (9 on-center, 26 off-center, and 15 on-off cells) to the first application of the agonist it appeared that the effective concentration range (threshold to saturation) was about 10 to 500 μM for carbachol and 0.5 to 5 mM for acetylcholine. No consistent differences in sensitivity to carbachol or acetylcholine were seen between the different types of ganglion cells.

Physostigmine. The effects of the cholinesterase inhibitor physostigmine were tested on 18 ganglion cells (4 on-center, 6 off-center and 8 on-off cells). 100 μM physostigmine caused a depolarization in darkness (2-17 mV) and a decrease in input resistance (25-120 MΩ). There was no noticeable difference between different types of ganglion cells. These results suggest that physostigmine potentiated a tonic, excitatory, cholinergic input that was active in darkness. It is

unlikely that the observed changes were due to a direct action of physostigmine on the ganglion cell membrane since it had no effect when transmitter release was blocked with cobalt. Physostigmine did not cause an increase in the amplitude of the light-evoked responses in any type of ganglion cell; like carbachol or acetylcholine, it usually caused a decrease in the amplitude of the light response.

Acetylcholine receptor antagonists

d-tubocurarine. The responses of ganglion cells to acetylcholine and carbachol were measured in the presence and absence of the nicotinic antagonist d-tubocurarine. It was not possible to make this comparison in a given cell, since cells usually did not respond to a second application of the agonist under any conditions. However, in the presence of 100 μM d-tubocurarine there was little or no response to the first application of carbachol or acetylcholine at concentrations which normally produced a response of about 20 mV, suggesting that the agonists were acting at nicotinic receptors. This result was observed in all of the 9 cells tested; in 5 of these cases 4 mM cobalt was also present.

Figure 4 shows the effect of d-tubocurarine on the synaptic input to an off-center ganglion cell; 100 μM d-tubocurarine caused a hyperpolarization and increase in resistance in darkness, and an increase in the size of the light-evoked response.
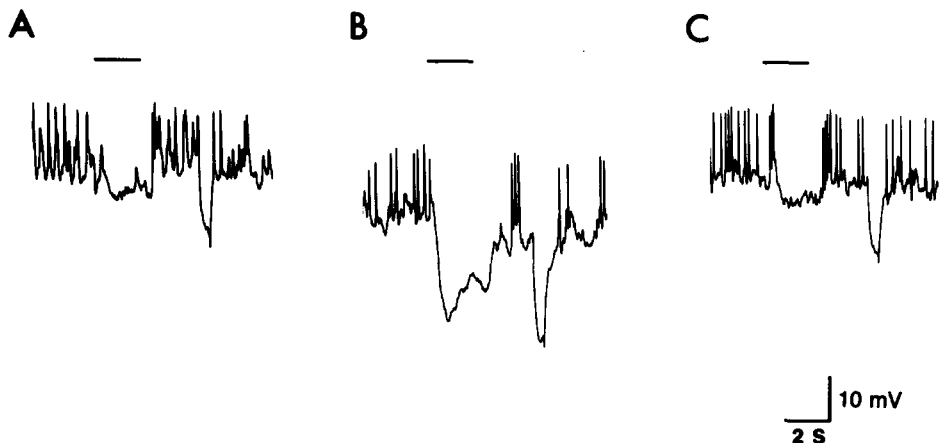

Fig. 4. Effect of d-tubocurarine on an off-center ganglion cell. A, in normal Ringer; B, in the presence of 100 μM d-tubocurarine; C, 5 min after return to normal Ringer solution. Light stimulus indicated by horizontal line above each trace. Light intensity -4.8, duration 2 sec. Downward deflection following each light response is the voltage drop produced by a -0.04 nA current pulse.

Current-voltage relations from an on-off ganglion cell are shown in Fig. 5. These measurements show the resistance of the cell in darkness in the presence (filled circles) and absence (open circles) of d-tubocurarine. In this cell d-tubocurarine caused a 9 mV hyperpolarization in darkness and increased the input resistance from 270 to 510 MΩ. The mean change in 27 ganglion cells (4 on-center, 11 off-center and 12 on-off cells) was a hyperpolarization of 4.7 mV and a resistance increase of 86 MΩ, with no obvious differences between cell types. Although in three of these cells there was no change in membrane potential, in no case did d-tubocurarine cause a depolarization. d-Tubocurarine had no effect on membrane potential or resistance in the presence of cobalt (n=6). These results suggest that all three classes of ganglion cells receive a tonic, nicotinic, cholinergic input in darkness. Both depolarizing and hyperpolarizing light responses were usually increased in amplitude in the presence of d-tubocurarine. This could have been due to the increased input resistance and/or actions of d-tubocurarine on presynaptic cells. The nicotinic antagonist mecamylamine (100 μM) was also tested on 4 cells; its effects were similar to those obtained with d-tubocurarine.

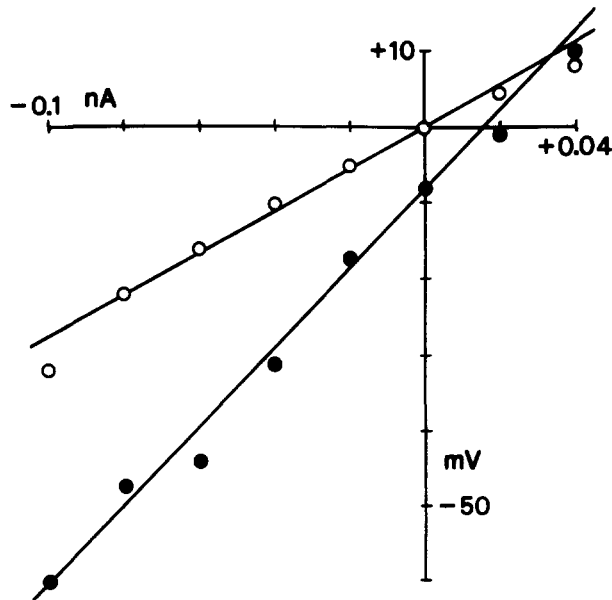Fig. 5. Current-voltage relations of an on-off ganglion cell in darkness in the absence (o) and presence (●) of 100 μM d-tubocurarine. Potential changes are plotted relative to control membrane potential in darkness.

Atropine. Responses to carbachol were not blocked by the muscarinic antagonist atropine (n=11, of which 9 were also in the presence of 4 mM cobalt). Figure 6 shows the effect of 3 mM atropine on the spontaneous and light-evoked activity of an off-center ganglion cell. In this cell 3 mM atropine caused a depolarization of the dark potential and an increase in resistance. It also caused an increase in the amplitude of the light-evoked response. Similar results were obtained with 1-3 mM atropine in 18 ganglion cells (4 on-center, 7 off-center and 7 on-off cells); the average depolarization of the dark potential was 5.3 mV and the average increase in input resistance was 51 MΩ. In 9 other ganglion cells atropine was applied in the presence of cobalt and had no effect on membrane potential or resistance. These results suggest that atropine caused a decrease in tonic inhibitory input. However, as discussed below, it is unlikely that the site of action of atropine was at receptors on ganglion cells.

The effects of both d-tubocurarine and atropine were reversed when they were removed from the superfusate.
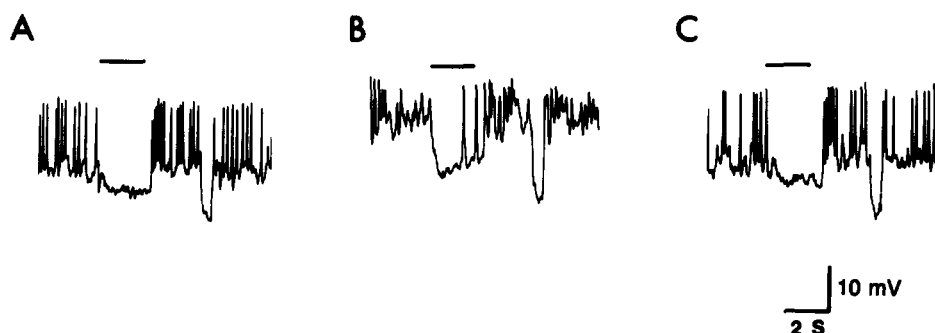

Fig. 6. Effect of atropine on an off-center ganglion cell. A, in normal Ringer; B, in the presence of 3 mM atropine; C, 5 min after return to normal Ringer solution. Light stimulus indicated by horizontal line above each trace. Light intensity -4.8, duration 2 sec. Downward deflection following each light response is the voltage drop produced by a -0.04 nA current pulse.

DISCUSSION

The finding that all three classes of ganglion cells were depolarized by low concentrations of carbachol, even when transmitter release was blocked with cobalt, indicates that they have receptors for acetylcholine. These receptors appear to be nicotinic since the action of carbachol was blocked by d-tubocurarine but not by atropine. However, this information alone does not es-

tablish that these receptors mediate any of the normal synaptic inputs to ganglion cells.

It has recently been shown that all classes of mudpuppy ganglion cells receive tonic excitatory and inhibitory synaptic inputs in darkness (14,16). The effects of physostigmine and d-tubocurarine on membrane potential and resistance in darkness suggest that at least part of the tonic excitatory input is via a cholinergic pathway. It is assumed that these agents acted at the nicotinic receptor sites on ganglion cells. However, it is possible that they acted at cholinergic sites elsewhere in the retina to alter non-cholinergic excitatory input to ganglion cells, in which case the acetylcholine receptors on the ganglion cells may have been non-functional receptor sites.

Although atropine caused a depolarization and increased resistance in darkness, it is unlikely that this was due to blocking an inhibitory cholinergic input to ganglion cells, since carbachol never caused a hyperpolarization of ganglion cells, even when its excitatory action was blocked by d-tubocurarine. An alternative explanation is that the depolarization produced by atropine was due to a presynaptic action, such as blocking excitatory input to a neuron which released a non-cholinergic inhibitory transmitter onto ganglion cells. At least some of the tonic inhibitory input to ganglion cells in darkness may be mediated by GABA, since receptors for this transmitter are present on all classes of ganglion cells (17,18) and bicucculine causes a depolarization in darkness in all classes of ganglion cells (unpublished observations). If atropine acted to reduce the activity of such an input, however, it might be expected that carbachol would also act at this site to increase the inhibitory input to ganglion cells. Therefore it is puzzling that no inhibitory action of carbachol was seen when its direct excitatory effect on ganglion cells was blocked with d-tubocurarine.

The fact that the light-evoked responses of ganglion cells were neither blocked by cholinergic antagonists nor enhanced by physostigmine suggests that they are not mediated by acetylcholine, which implies that the tonic cholinergic input was not strongly driven by the light stimuli used in these experiments. Although not described above, responses to illumination of the receptive field surround were also not blocked by the cholinergic antagonists. In rabbit retina some ganglion cells appear to receive a cholinergic excitatory input that is not modulated by light (4,5) but this has been attributed to a light-independent component of acetylcholine release by neurons in which there is also a light-dependent release of acetylcholine (6). It has been suggested that acetylcholine is mainly involved in transient responses or responses to moving stimuli (5,6,19), but the transient responses of mudpuppy ganglion cells were not blocked by d-tubocurarine. The effects of acetylcholine antagonists on the responses of mud-

puppy ganglion cells to moving stimuli have not been investigated. It would also be of interest to study their effects on the responses of directionally selective ganglion cells in mudpuppy. If a truly light-independent synaptic input exists, one might expect the presynaptic neuron to have little or no response to light flashes. The membrane potential of such a cell might be controlled by other factors, such as the state of adaptation.

In summary, there appears to be tonic cholinergic excitatory input to all types of ganglion cells in the mudpuppy retina, but the specific kinds of light stimuli which modulate this input are not known.

REFERENCES

1. Neal, MJ (1983) In: Osborne N, Chader G (eds) Progress in Retinal Research, Vol. 4. Pergamon, New York, pp 191-212

2. Puro DG (1985) In: Morgan WW (ed) Retinal Transmitters and Modulators: Models for the Brain, Vol. 1. CRC Press, Boca Raton, pp 63-91

3. Masland RH, Ames A (1976) J Neurophysiol 39: 1220-1235

4. Ariel M, Daw NW (1982) J Physiol 324: 135-160

5. Ariel M, Daw NW (1982) J Physiol 324: 161-185

6. Masland RH, Mills JW, Cassidy C (1984) Proc R Soc Lond B 223: 121-139

7. Masland RH, Livingstone CJ (1976) J Neurophysiol 39: 1210-1219

8. Massey SC, Neal MJ (1979) J Neurochem 32: 1327-1329

9. Straschill M, Perwein J (1973) Pflügers Arch 339: 289-298

10. Ikeda H, Sheardown MJ (1982) Neuroscience 7: 1299-1308

11. Wässle H, Voigt T, Schmidt M, Humphrey M (1986) Neuroscience Res 3:

12. Negishi K, Kato S, Teranishi T, Laufer M (1978) Brain Res 148: 85-93

13. Glickman RD, Adolph AR, Dowling JE (1982) Brain Res 234: 81-89

14. Belgum JH, Dvorak DR, McReynolds JS (1982) J Physiol 326: 91-108

15. Fain GL (1975) J Physiol 252: 735-769

16. Belgum JH, Dvorak DR, McReynolds JS (1983) J Physiol 340: 599-610

17. Miller RF, Frumkes TE, Slaughter M, Dacheux RF (1981) J Neurophysiol 45: 764-782

18. Belgum JH, Dvorak DR, McReynolds JS (1984) J Physiol 354: 273-286

19. Famiglietti EV Jr (1983) Vision Res 23: 1265-1279