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GABA-ANTAGONISTS ALTER SPATIAL SUMMATION IN RECEPTIVE FIELD CENTRES OF ROD- BUT NOT CONE-DRIVEN CAT RETINAL GANGLION Y-CELLS

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SUMMARY

1. When Y-type cat retinal ganglion cells were driven by cones, the I.V. administration of the GABA-antagonist picrotoxin failed to alter receptive field centre size.

2. This result is in marked contrast to our previous finding that when Y-cells were driven by rods, GABA-antagonists led to specific and reversible changes in centre size.

3. These results taken together suggest that for centre signals of Y-cells, the rod and cone pathways are pharmacologically distinct.

INTRODUCTION

Responses of X- and Y-type cat retinal ganglion cells (Enroth-Cugell & Robson, 1966) are known to be differentially affected by antagonists of putative neurotransmitters (Kirby & Enroth-Cugell, 1976; Kirby, 1979). For Y-cells there is a shift in centre-surround balance in favour of the centre following I.V. administration of GABA-antagonists (bicuculline and picrotoxin), while responses of X-cells remain virtually unaffected (Kirby & Enroth-Cugell, 1976). Similarly for X-cells, there is a shift in centre-surround balance in favour of the centre following administration of the glycine antagonist strychnine, while responses of Y-cells remain minimally affected (Kirby, 1979). In addition, a more recent study has shown that GABA-antagonists have opposite effects on centre size in on-and off-centre Y-cells: for on-centre cells centre size decreases while for off-centre cells, it increases (Kirby & Schweitzer-Tong, 1981). It is important to note that all of these pharmacological experiments were done under scotopic conditions. Because rod and cone signals are likely to have different pathways through the cat retina (Kolb, 1979), in this paper we have examined the effect of picrotoxin on receptive field center size in cone-driven Y-cells and compared these results with those obtained under scotopic conditions.

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METHODS

Preparation and recording. Experimental methods have previously been described in detail (Kirby & Schweitzer-Tong, 1981) and are only briefly given here. Anaesthesia was initiated with halothane or ketamine hydrochloride, continued during surgery with sodium thiamylal and maintained for the remainder of the experiment with ethyl carbamate. The cervical sympathetic trunk was cut bilaterally in all cats but one. Continuous I.V. infusion of gallamine triethiodide was used for muscle paralysis. Subscapular temperature was maintained at 38 °C, mean arterial blood pressure above 90 mmHg and end-expired CO_2 at about 4% by adjusting the minute volume of the respiration pump. Retinal ganglion cell activity was recorded from the optic tract with tungsten-in-glass electrodes (Levick, 1972).

Stimulation. A Maxwellian view optical system with neutral density and colour filters was used to elicit either rod-driven (scotopic) or cone-driven (photopic) responses from ganglion cells (Enroth-Cugell, Hertz & Lennie, 1977). *Scotopic* ganglion cell responses were obtained with green stimulus spots (Ilford 603 or 623, dominant wave-length 490 nm) against weak red backgrounds (Ilford 608, transmits above 620 nm) or no background; *photopic* responses with red stimulus spots (Ilford 608) against a strong green background (Ilford 603 or 623). In three cells the two-filter method of Wald (1960) was used to verify that the change in stimulus conditions resulted in a Purkinje shift.

X/Y-cell classification. Classification was based on responses to phase-reversed or flashed stationary sinusoidal grating patterns generated on an oscilloscope (Enroth-Cugell & Robson, 1966; Hochstein & Shapley, 1976). The mean luminance of the oscilloscope screen (P31 phosphor) was 1·1 log scotopic cd/m^2. The cat viewed the oscilloscope through 4 mm artificial pupils placed in front of a clear contact lens of suitable power. When the Maxwellian view stimulator was used the artificial pupil was removed.

Area-sensitivity curves. Receptive field centre size was estimated from area-sensitivity curves (Cleland & Enroth-Cugell, 1968; Kirby & Schweitzer-Tong, 1981) obtained by finding the illumination needed to elicit a small, 'threshold' response to a series of centred, concentric circular spots of increasing diameter turned on and off at 4 Hz. Thresholds were routinely determined by listening; however we have previously found (Kirby & Schweitzer-Tong, 1981) that these thresholds and those calculated by computer are in good agreement.

An integrated Gaussian curve was fit to the experimental area-sensitivity data and an index of centre size ('the equivalent centre diameter') was calculated from the parameters of the best fitting integrated Gaussian. A detailed description of the rationale and the procedure has been given previously (Kirby & Schweitzer-Tong, 1981).

It is perhaps worth pointing out that a change in equivalent centre diameter following drug administration cannot be ascribed to a simple over-all sensitivity change such as that produced by placing a neutral density filter in front of the eye. This can only produce a vertical displacement of the area-sensitivity curve as a *whole* which clearly does not produce a change in equivalent diameter. On the other hand, provided that small spot sensitivity remains unaltered after drug administration, sensitivity to large spots (i.e. asymptotic sensitivity) *must* change if there is either an increase or a decrease in equivalent centre diameter. In fact, our scotopic results show that small spot sensitivity does not change much (on-centre cells $\bar{x} = -0{\cdot}13$ log units, off-centre $\bar{x} = -0{\cdot}04$), whereas large spot sensitivity increases for off-centre cells ($\bar{x} = +0{\cdot}26$) and decreases for on-centre cells ($\bar{x} = -0{\cdot}38$). Moreover, the change in large spot sensitivity is highly correlated with relative change in equivalent diameter (on-centre $r = 0{\cdot}99$, off-centre $r = 0{\cdot}98$).

Experimental protocol. The general strategy of these experiments was to measure receptive field centre size *before* and at various intervals *after* the administration of the GABA-antagonist. Once a Y-cell had been isolated one or two area-sensitivity curves were obtained before the administration of the drug. As soon as this was done 0·2–0·4 mg/kg picrotoxin (Sigma Chemical Co., St. Louis, Mo.) was given I.V. over 2 min. Area-sensitivity curves were then measured as many times as possible over a period of at least 30 min, or if there was a change in field size until the cell had recovered to its initial condition, or was lost. Four to five minutes were needed to complete one area-sensitivity curve. Before each set of measurements a sensitivity profile of the centre was obtained (Cleland & Enroth-Cugell, 1968; Kirby & Schweitzer-Tong, 1981). Whenever a repeat profile indicated that the eye had moved, the stimulus was recentred.

RESULTS

Area–sensitivity curves were obtained from eight cells under conditions which ensured that they were driven by cones. In no case was picrotoxin administration followed by a significant change in equivalent centre diameter. This is in marked contrast to the changes we have previously reported for twenty-five out of twenty-six rod-driven Y-cells (Kirby & Schweitzer-Tong, 1981), where the administration of the

TABLE 1. Summary of changes in equivalent centre diameter of cat retinal ganglion Y-cells following the administration of GABA-antagonists. The scotopic results which show statistically significant changes (paired *t* test) in equivalent centre diameter are from experiments reported elsewhere (Kirby & Schweitzer-Tong, 1981). The photopic results are within the range of values obtained in control experiments.

Condition	Average change in equivalent centre diameter (degrees of visual angle) Initial diameter − post-drug diameter
Scotopic	
$n = 12$ on-centre	−0·66° (26 %)*
$n = 14$ off-centre	+1·20° (46 %)*
Photopic	
$n = 5$ on-centre	+0·04° (1·4 %)
$n = 3$ off-centre	+0·04° (1·9 %)
Control	
(measurement variability)	Initial (1)–initial (2)
$n = 12$	−0·06° (3 %)

* $P < 0{\cdot}01$.

GABA-antagonists picrotoxin and bicuculline produced significant, reversible changes in equivalent centre diameter. Table 1 summarizes the findings for the scotopic and the current photopic experiments, as well as *control* experiments which assessed the average variability between repeated determinations of the equivalent centre diameter before drug administration. Under scotopic conditions, the change in equivalent centre diameter for on-centre cells was an average *reduction* of 0·66°, 26 % change; for off-centre cells the change was an average increase of 1·2°, a 46 % change. Under photopic conditions, for either on- or off-centre cells, the average difference between initial and post-drug equivalent centre diameters was not significant.

To rule out the possibility that our sample of cone-driven cells was insensitive to picrotoxin at all adaptation levels, or that the picrotoxin had lost its potency, we studied individual cells under both scotopic and photopic conditions. For one on- and one off-centre cell we succeeded in completing the experiment under both adaptation conditions. The on-centre cell was studied at the scotopic and then at the photopic level; while the off-centre cell was studied in the reverse order. The results are shown in Fig. 1 for the off-centre cell studied first under photopic conditions.

The photopic equivalent centre diameter prior to drug administration was 1·91° (▲). At 4·5 min after the administration of picrotoxin the equivalent centre was 1·84° (△) and failed to show a significant change in the next 80 min (the difference of 0·07° is a change of 4 %).

Following dark adaptation, the experiment was repeated under scotopic conditions. Initially, the equivalent centre diameter was 3·65° (●) but 4·5 min after the administration of picrotoxin, the equivalent diameter had expanded to 4·27° (○) which is a 0·62° (17 %) change in diameter. By 26·5 min, the equivalent diameter had returned to its initial dark adapted value of 3·65° (□).

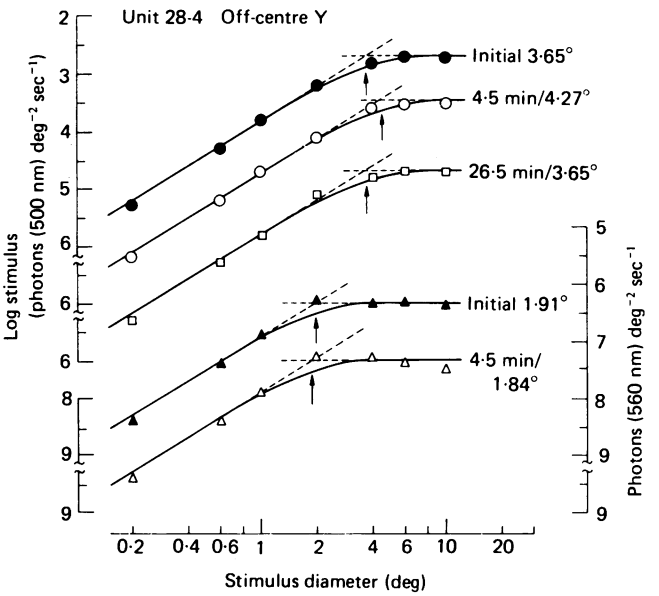

Fig. 1. Effect of picrotoxin on area–sensitivity curves of an off-centre ganglion cell studied first under photopic, then under scotopic conditions. Triangles (△, ▲) represent data collected when the cell was cone-driven (the photopic ordinate is on the right-hand side), circles (○, ●) and squares (□) represent area-sensitivity data collected when the cell was rod-driven (the scotopic ordinate is on the left-hand side). The continuous curves drawn to the data are the best fitting (least-squres criterion) integrated Gaussian curves and the vertical arrows, at the intersection of the two dashed lines asymptotic to the curves, mark the *equivalent centre diameter*. With the exception of the initial scotopic and photopic curves, each curve has been shifted down by 1 or 2 log units.

Fig. 2 shows the results of the analogous experiment performed in the reverse order with an on-centre Y-cell. As illustrated by the top two area–threshold curves, the scotopic equivalent centre diameter decreased from 2·29° (●) to 1·98° (○), 4·5 min after picrotoxin administration. This change in equivalent diameter (0·31, a 13·5 % change) was fully reversible. At 28 min after drug administration, the equivalent centre diameter had returned to its initial dark adapted value of 2·29° (□). Under photopic conditions, the equivalent centre diameter was 1·64° initially (▲) and remained unchanged at 4·5 min following picrotoxin administration (△). At 9 and at 23·5 min (not shown in the Figure) the equivalent diameter was still essentially unchanged (1·64° and 1·70° respectively).

For the ganglion cells shown in Figs. 1 and 2, the photopic equivalent diameter is smaller than the scotopic one. However, we have data from three additional cells which agree with a previous study (Enroth-Cugell *et al.* 1977), in showing no systematic difference in centre size with a change from scotopic to photopic

conditions: for one cell the photopic centre was smaller than the scotopic one, for the other two the photopic centre was larger.

DISCUSSION

Our results clearly show that the administration of GABA-antagonists alters equivalent centre size when cells are driven by rods, but fails to alter centre size when cells are driven by cones.

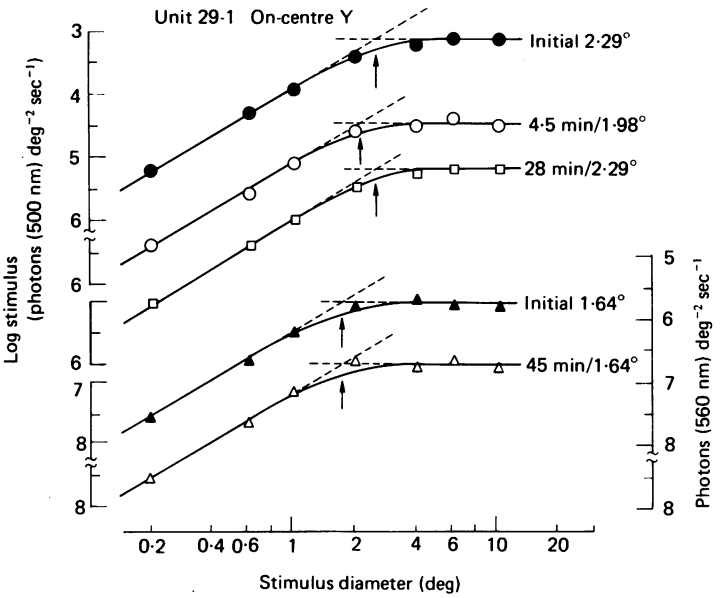

Fig. 2. Effect of picrotoxin administration on area–sensitivity curves of an on-centre cell studied first under scotopic, then under photopic conditions. Symbols follow the conventions described for Fig. 1.

We assume that picrotoxin and bicuculline antagonize activity post-synaptic to amacrine cells because autoradiographic studies have localized GABA to subpopulations of amacrine cells (Marshall & Voaden, 1975; Nakamura, McGuire & Sterling, 1978; Pourcho, 1980). In fact, the *differential* effects of GABA-antagonists at scotopic and photopic levels may be due to the absence of such amacrine intermediaries in the cone pathway. Whereas rod bipolars have never been observed to synapse with ganglion cells but always appear to utilize at least one amacrine intermediary, cone bipolars have been observed to synapse directly with ganglion cells (Kolb, 1979).

One observation that renders this account less attractive is that cones appear to receive substantial rod input (Nelson, 1977; Nelson, Kolb, Famiglietti & Gouras, 1976), suggesting a pathway for rod signals by means of which the rod-bipolar-amacrine-ganglion cell circuits may be bypassed. Also, we know that some GABA circuits are active under photopic conditions because the sensitivity of Y-cell non-linear subunits is reduced by picrotoxin at photopic levels (Linsenmeier & Frishman, 1980); but as our current results show, these subunit circuits cannot be

involved in transmitting *centre* signals since GABA-antagonists fail to alter centre size at photopic levels. Thus, although GABA does mediate some cone-driven Y-cell responses, it does not seem to mediate *centre signals*, which must therefore reach Y-cells either directly through cone-bipolars or through a bipolar-amacrine-pathway that does not utilize GABA.

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