Agonist-induced glycogenolysis in rabbit retinal slices and cultures

Hani Ghazi & ¹Neville N. Osborne

Nuffield Lab. of Ophthalmology, Walton Str., Oxford OX2 6AW

- 1 The effects of different putative retinal transmitters and/or modulators on glycogenolysis in rabbit retinal slices and in retinal Müller cell cultures were examined.
- 2 Incubation of rabbit retinal slices or primary retinal cultures (either 3–5 day-old or 25–30 day-old) in a buffer solution containing [³H]-glucose resulted in the accumulation of newly synthesized [³H]-glycogen.
- 3 Noradrenaline (NA), isoprenaline, vasoactive intestinal peptide (VIP), 5-hydroxytryptamine (5-HT) and 8-hydroxy-dipropylaminetetralin (8-OH-DPAT) stimulated the hydrolysis of this newly formed ³H-polymer. The potency order of maximal stimulations was: VIP > NA > isoprenaline > 5-HT > 8-OH-DPAT.
- 4 The putative retinal transmitters, dopamine, γ -aminobutyric acid (GABA), glycine and taurine and the muscarinic agonist carbachol (CCh) had no effect on [³H]-glycogen content.
- 5 The glycogenolytic effects of NA/isoprenaline and 5-HT/8-OH-DPAT appear to be mediated by β -adrenoceptors and 5-HT₁ receptors (possibly 5-HT_{1A}), respectively while the VIP-induced response involved another receptor subtype.
- 6 Agonists which mediated [³H]-glycogen hydrolysis also stimulated an increase in adenosine 3':5'-cyclic monophosphate (cyclic AMP) formation. Both responses are blocked to a similar extent by the same antagonists and so are probably mediated via the same receptor subtypes. Moreover, dibutyl cyclic AMP (db cyclic AMP) promoted tritiated glycogen breakdown in the three retinal preparations.
- 7 Not all receptors linked to cyclic AMP production however promote glycogenolysis. Dopamine and apomorphine stimulated cyclic AMP formation via D₁-receptors without influencing glycogenolysis. These receptors are exclusively associated with neurones.

Introduction

Glycogen is the major energy reserve in the avascular rabbit retina (Kuwabara & Cogan, 1961; Mizuno & Sata, 1975). Histochemical studies on the rabbit retina showed that glycogen stores are predominantly associated with Müller cells (Kuwabara & Cogan, 1961; Mizuno & Sata, 1975). Furthermore, these are major sites for glycogen synthesis (Kuwabara & Cogan, 1961). It is known that a number of neurotransmitters including neuropeptides induce glycogen breakdown in mouse cerebral cortex (Magistretti *et al.*, 1981; 1984; 1986; Quach *et al.*, 1978; 1980; 1982), astrocytic cultures

(Magistretti *et al.*, 1983), leech segmental ganglia (Seal & Pentreath, 1985) and in cultured glial cells of the chick retina (Koh *et al.*, 1984). Recent evidence has indicated that transmitter-like substances induce their glycogenolytic effects in the CNS by stimulating adenosine 3':5'-cyclic monophosphate (cyclic AMP) production (Magistretti *et al.*, 1983; 1986).

The purpose of the present study was to investigate the effects of different putative retinal transmitters and/or modulators on glycogenolysis in rabbit retinal slices and to correlate these actions with the ability of the individual substances to stimulate cyclic AMP production. Experiments were also carried out on retinal cultures containing primarily

¹ Author for correspondence.

Müller cells (Osborne *et al.*, 1988; Ghazi & Osborne, 1988a) to determine the cellular site of glycogenolysis.

Methods

Adult albino rabbits (2–3 kg) were obtained from the University Breeding Farm. These rabbits had free access to food and water while being maintained at a temperature of 18–20°C.

Intact retina

Agonist-induced stimulation of glycogenolysis Rabbits were anaesthetised with an intramuscular injection of Hypnorm (0.5 ml kg⁻¹) and killed by an overdose injection of sodium pentobarbitone. Eyes were immediately enucleated and the retinas quickly dissected and washed in oxygenated modified Krebs-Ringer bicarbonate medium (composition, mM: NaCl 120, KCl 5, CaCl₂ 2.6, MgSO₄ 0.67, KH₂PO₄ 1.2, glucose 5.5 and NaHCO₃ 27.5) at pH 7.4. Retinal slices (0.25 × 0.25 mm thick) were obtained with a McIlwain tissue chopper, suspended in Krebs-Ringer solution and preincubated for 15 min at 37°C under a constant stream of 95% O₂:5% CO₂. The slices were then washed with fresh buffer and resuspended in oxygenated buffer (2.5 ml per retina). Aliquots of the tissue slices suspension (0.29 ml) were pipetted into individual tubes to which were added 0.01 ml of a buffer solution containing [³H]-glucose (final conc. 50 nM). The tubes were then incubated for 30 min at 37°C in a shaking water bath. Buffer (10 µl) with or without antagonist was added to each tube and the incubation continued for 5 min. Following this period 0.01 ml of drug or buffer was added to individual samples and the incubation continued for 30 min at 37°C under gentle agitation. The reactions were stopped by rapid centrifugation (1000 g for 5 min). The supernatants were discarded, replaced with fresh buffer and the slices immediately sonicated for about 9 s. An aliquot (approx. 0.25 ml) of each of the resulting homogenates was quickly transferred to another tube and placed in boiling water for 10 min, followed by a short centrifugation. The supernatants of these samples were assayed for [³H]-glycogen content. Another sample of each of the homogenates was taken for determination of protein content by the method of Bradford (1976).

An ethanol filter paper technique (Solling & Esmann, 1975) was used to isolate and assay for [³H]-glycogen. Briefly, 0.15 ml aliquots of the deproteinized supernatant were spotted on filter paper discs, air dried and then immersed in approximately 10 ml of ice-cold 60% ethanol/10% trichloroacetic

acid for 10 min. This solution was replaced with 66% ethanol at room temperature for 10 min. This procedure was repeated 6 times. The filter discs were then placed in acetone for 5 min and dried for 20 min. Each disc was subsequently placed in a scintillation counting vial and the [³H]-glycogen extracted by the addition of 2 ml of boiling water. After cooling, 10 ml of Insta-gel scintillation fluid were added and the radioactivity determined in a liquid scintillation counter (Wallac 1211 Rackbeta Liquid Scintillation Counter, LKB, Finland).

Control experiments showed that radioactivity of [³H]-glucose spotted onto filter paper discs was not carried through to provide a source of error. Moreover, treating samples of the supernatant before the glycogen extraction procedure, with or without 4 units ml⁻¹ of amylo-1-6-glucosidase demonstrated that as little as 3.0% of the ³H-isolated product was not tritiated glycogen. Since it is known that amylo-1-6-glucosidase hydrolyzes glycogen to form glucose (Nahorski & Rogers, 1972; Passonneau & Lauderdale, 1974) the contamination may be glucose-containing gangliosides and/or cerebroside (Nahorski & Rogers, 1972).

Agonist-induced stimulation of cyclic AMP production With minor modifications, the method described by Kebejian *et al.* (1972) was used to stimulate cyclic AMP production by the various agents in homogenates of rabbit retinas. The retinas were dissected, weighed and quickly homogenized in an ice-cold solution of a Tris I buffer (0.02 ml per mg of wet weight). Tris I buffer consists of: Tris 2 mM, maleate 2 mM and EGTA 2 mM; pH 7.4. Aliquots of 0.13 ml of Tris II buffer solution [Tris II buffer consists of: Tris 80 mM, MgSO₄ 2 mM, theophylline 10 mM or isobutylmethylxanthine (IBMX) 1 mM, EGTA 0.2 mM; pH 7.4] and 0.01 ml of the agonist (made up in Tris II buffer) were added to assay tubes in triplicate. Aliquots of 0.02 ml of retinal homogenates were then added to each of the tubes. When antagonists were used, 0.01 ml of the antagonist solution replaced 0.01 ml of Tris II buffer. Basal levels of cyclic AMP in retinal homogenates were determined in assay tubes containing only the homogenate and 0.14 ml of Tris II buffer. Stimulation of cyclic AMP production was initiated by adding 0.05 ml of ATP (made up in Tris II buffer) in each assay tube. The tubes were incubated for 5 min at 37°C in a gently shaking water bath. The reaction was stopped by placing the tubes in boiling water for 3 min, followed by centrifugation at 11,500 g for 5 min. Aliquots (0.05 ml) of the supernatant were removed and kept frozen before being assayed for cyclic AMP content. The method of Brown *et al.* (1972), which is based on a competition between [³H]-cyclic AMP and unlabelled cyclic AMP for specific binding sites to a

Figure 1 Time course of the synthesis of [^3H]-glycogen in rabbit retinal slices and of its hydrolysis by various agents. Slices were incubated in a modified Krebs-bicarbonate solution (see Methods section) containing [^3H]-glucose and the content of [^3H]-glycogen determined at the appropriate times as described in the Methods section. After 35 min of incubation, either drugs or buffer solutions were added (arrow) and the reaction was stopped at increasing intervals and the amount of [^3H]-glycogen determined. Points are means $\pm$ s.e. (error bars) from 4 experiments. Assay conditions and presentation of results are as described in Table 2. Noradrenaline (Δ); isoprenaline ($\blacksquare$); 5-hydroxytryptamine ($\blacktriangle$); vasoactive intestinal peptide ($\circ$) and 8-hydroxy-dipropylaminetetralin ($\square$).

binding protein was used to quantify cyclic AMP levels.

Preparation of retinal cultures

Retinas from 1 to 5 day postnatal rabbit pups were dissociated by a modified trypsinisation procedure as previously described (Beale *et al.*, 1982; Osborne & Beaton, 1986). The dissociated cells were suspended in culture medium consisting of 85% Eagles Minimal Essential medium with Earle's salt (Gibco). The cells in 0.5 ml of culture medium were placed on a 13 mm diameter glass coverslip coated with poly-L-lysine. The coverslips were in Multidish (Falcon 3008) wells and the density of the cells was between 4000–5000 per mm^2 . The cultures were grown at 35.5°C in a humidified atmosphere containing 5% CO_2 . The culture medium was changed after 3 days and thereafter at 2- to 3-day intervals.

Agonist-induced stimulation of [^3H]-glycogen hydrolysis Retinal cultures corresponding to between 3–5 and 25–30 days following seeding, were washed twice with previously oxygenated Krebs-Ringer bicarbonate buffer (pH 7.4): 0.8 ml of this buffer solution containing [^3H]-glucose (final conc. 50 nM) was added to each well and the cultures were incubated for 70 min (3–5 day) or 85 min (25–30 day) in a humidified 95% O_2 :5% CO_2 atmosphere at

35.5°C. Either antagonist or buffer (0.1 ml of either) was added to each culture well and the incubation continued for 5 min. These incubation times were used since time course experiments indicated that the rate of [^3H]-glucose incorporation into glycogen decreased significantly at 75 min (3–5 day) and 90 min (25–30 day), to eventually reach a plateau. Each well then received another 0.1 ml of drug or buffer and the incubation was continued for 30 min at 35.5°C in the same humidified atmosphere. The reactions were terminated by thrice washing the cultures with buffer, removing the coverslips and placing them in a plastic vial containing 0.5 ml of fresh buffer. The coverslips were then crushed, vortexed, sonicated for 20 s and placed in boiling water for 10 min, followed by a short centrifugation (1000 *g* for 5 min). The supernatant of each sample was analysed for its content of [^3H]-glycogen in the same manner as for retinal slices, and the protein content was determined by the method of Bradford (1976).

Agonist-induced stimulation of cyclic AMP production The cultures were washed twice with culture medium and incubated for 5 min in Eagles Minimal Essential medium containing Tris 2 mM, maleate 2 mM, EGTA 2 mM; pH 7.4; (Tris I/MEM). Two coverslips containing the cultures were then transferred to individual plastic tubes already containing 0.14 ml of Tris II/MEM (MEM, Tris 80 mM, EGTA 0.2 mM, theophylline 10 mM or IBMX 1 mM, MgSO_4 2 mM; pH 7.4) and 0.01 ml of antagonist (made up in Tris II/MEM) where appropriate. Following an incubation for 5 min, 0.01 ml of the agonist (made up in Tris II/MEM) was added to each tube followed by 0.05 ml of ATP (made up in Tris II/MEM) to initiate the reaction. The tubes were then incubated for 5 min in a shaking water bath at 37°C. Control tubes contained no agonist or antagonist and all experiments were carried out in triplicate. Following centrifugation, cyclic AMP was determined in 0.05 ml aliquots of the supernatants.

Materials

D-[6- ^3H]-glucose (30.2 Ci mmol^{-1}) and [8- ^3H]-cyclic AMP (41.7 Ci mmol^{-1}) were purchased from Amersham International plc, UK. Prazosin was obtained from Pfizer, UK; ketanserin and Hypnorm (a central depressant) from Janssen Pharmaceuticals, Germany; methysergide, spiroxatrine and mianserin from Sandoz, Switzerland; vasoactive intestinal peptide (VIP) from Bachem U.K. Ltd. MDL-72222 (1 αH ,3 α ,5 αH -tropan-3-yl-3,5-dichlorobenzoate) was a gift from Dr Fozard (Merrell Dow) and sodium pentobarbitone was from Willows Francis Veterinary, West Sussex, England. RU24696 (5-methoxy-

3(1,2,3,6-tetrahydropyridin-4-yl)1H indole) was obtained from Le Centre de Recherche Roussel UCLAF. All other chemicals and drugs were purchased from Sigma, U.K.

Statistical analysis

Statistical significance was determined by the Mann-Whitney test. A *P* value of <0.05 was considered significant.

Results

Retinal slices

Rabbit retinal slices incubated in the presence of [³H]-glucose (50 nM) for different time periods, followed by the determination of [³H]-glycogen content demonstrated that the accumulation of newly synthesized [³H]-glycogen was linear for the first 30 min. Thereafter a plateau was reached and maintained for at least 60 min (Figure 1).

Retinal slices which had been preincubated for 35 min with [³H]-glucose and then exposed to 500 μM noradrenaline (NA), isoprenaline, 5-HT, 8-OH-DPAT or 5 μM VIP resulted in maximal increases in the hydrolysis of [³H]-glycogen (see Table 1). All the agents produced a rapid rate of decrease in [³H]-glycogen content, though NA, isoprenaline and VIP induced a much more pronounced decrease than 5-HT or 8-OH-DPAT. Dibutyl cyclic AMP at 250 μM also induced a substantial reduction in [³H]-glycogen content (Table

Figure 2 Dose-response curves of [³H]-glycogen hydrolysis elicited by noradrenaline (●), isoprenaline (□) and vasoactive intestinal peptide (Δ) in rabbit retinal slices. Conditions of the experiments are as described in Table 2 except that antagonists were not used. Points represent means ± s.e. (error bars) from 4 separate experiments.

1). Figures 2 and 3 demonstrate that the glycogenolytic effects of the agonists were dose-dependent resulting in EC₅₀ values of 22.7 nM (NA), 27.9 nM (isoprenaline), 0.422 nM (VIP), 306 nM (5-HT) and 194 nM (8-OH-DPAT). Dopamine, CCh, GABA, glycine and taurine did not induce glycogenolysis in retinal slices (Table 1).

Prazosin (10 μM), yohimbine (10 μM) or phentolamine (10 μM), three specific α-adrenoceptor antagonists, and spiroxatrine (10 μM), a 5-HT_{1A} receptor antagonist (Nelson & Taylor, 1986; Göthert & Schli-

Table 1 Effect of various agents on % of [³H]-glycogen hydrolysis (glycogenolysis) in rabbit retinal slices or % stimulation of cyclic AMP production over basal levels in retinal homogenates

Agent	Conc (μM)	% [³ H]-glycogen hydrolysis	% cyclic AMP production
Buffer	—	0	0
NA	500	46.33 ± 1.62	269.63 ± 13.35
Isoprenaline	500	42.01 ± 0.90	114.44 ± 6.34
VIP	5	49.98 ± 3.89	357.88 ± 43.01
5-HT	500	37.37 ± 1.95	139.02 ± 7.39
8-OH-DPAT	500	31.81 ± 2.30	128.86 ± 10.07
GABA	500	5.65 ± 2.58 NS	9.10 ± 2.61 NS
Glycine	500	7.42 ± 1.33 NS	7.83 ± 2.14 NS
Taurine	500	5.22 ± 2.32 NS	5.08 ± 3.87 NS
Dopamine	500	11.43 ± 1.36 NS	199.82 ± 8.56
Apomorphine	500	4.94 ± 0.99 NS	184.57 ± 13.81
Carbachol	500	7.40 ± 6.49 NS	-24.78 ± 8.66
db cyclic AMP	250	62.13 ± 1.28	

Values are means ± s.e.mean where *n* is from 6–14 separate experiments. NA = noradrenaline, VIP = vasoactive intestinal peptide, 8-OH-DPAT = 8-hydroxy-dipropylaminetetralin, GABA = γ-aminobutyric acid, and db cyclic AMP = dibutyl cyclic AMP. NS = insignificant compared with controls.

Figure 3 Dose-response curves of $[^3\text{H}]$ -glycogen hydrolysis elicited by 5-hydroxytryptamine (●) and 8-hydroxy-dipropylaminetetralin (□) in rabbit retinal slices. Conditions of the experiments are as described in Table 2 except that antagonists were not added. Points represent means $\pm$ s.e. (error bars) from 4 separate experiments.

ker, 1987), had no effect on either the NA- (Table 2) or isoprenaline-induced glycogenolytic effect. In contrast, propranolol ($1\text{ }\mu\text{M}$), a β -adrenoceptor blocker, which had no significant action of $[^3\text{H}]$ -glycogen content strongly inhibited both the NA- and isoprenaline-induced responses (Table 2). The effects of the dopamine receptor antagonists, haloperidol, spiroperidol and (+) and (-)-butaclamol ($10\text{ }\mu\text{M}$), on the responses induced by the two agonists were tested. It was found that these antagonists had no effect on the isoprenaline-induced response, however the NA-induced response was slightly inhibited by haloperidol but not by either (+) and (-)-butaclamol or spiroperidol. This is interesting since neither dopamine nor apomorphine stimulated glycogenolysis in retinal cells, but strongly stimulated the production of cyclic AMP (Table 1). It is therefore concluded that NA and isoprenaline influenced β -adrenoceptors to promote glycogenolysis in the

Table 2 Effect of various antagonists on noradrenaline/isoprenaline-induced $[^3\text{H}]$ -glycogen hydrolysis or cyclic AMP production

Antagonist Agent	added	% $[^3\text{H}]$ -glycogen hydrolysis	% cyclic AMP production
Buffer		0	0
NA (500 μM)		44.87 ± 3.05	266.81 ± 13.02
NA	Propranolol ($1\text{ }\mu\text{M}$)	$*10.46 \pm 1.55$	$*150.22 \pm 10.41$
NA	Propranolol ($10\text{ }\mu\text{M}$)	$*7.48 \pm 1.14$	$*107.26 \pm 5.54$
NA	Haloperidol ($1\text{ }\mu\text{M}$)	$*25.71 \pm 4.15$	$*87.14 \pm 3.29$
NA	Spiroperidol ($1\text{ }\mu\text{M}$)	NS 44.08 ± 2.09	$*155.12 \pm 2.63$
NA	(+)-Butaclamol ($1\text{ }\mu\text{M}$)	NS 40.85 ± 1.85	$*151.63 \pm 12.54$
NA	(-)-Butaclamol ($1\text{ }\mu\text{M}$)	NS 44.83 ± 2.96	NS 255.82 ± 5.31
NA	Prazosin ($10\text{ }\mu\text{M}$)	NS 43.43 ± 1.03	NS 263.96 ± 10.25
NA	Yohimbine ($10\text{ }\mu\text{M}$)	NS 43.50 ± 2.99	NS 270.03 ± 15.10
NA	Phentolamine ($10\text{ }\mu\text{M}$)	NS 44.90 ± 1.16	NS 259.97 ± 11.71
NA	Spiroxatrine ($10\text{ }\mu\text{M}$)	NS 43.71 ± 0.85	NS 272.06 ± 9.91
Isoprenaline (500 μM)		40.63 ± 5.26	114.40 ± 6.32
Isoprenaline	Propranolol ($1\text{ }\mu\text{M}$)	$*5.22 \pm 1.38$	$*14.62 \pm 6.48$
Isoprenaline	Propranolol ($10\text{ }\mu\text{M}$)	$*1.92 \pm 1.03$	$*5.18 \pm 2.33$
Isoprenaline	Haloperidol ($1\text{ }\mu\text{M}$)	NS 41.65 ± 3.80	NS 109.93 ± 4.11
Isoprenaline	Spiroperidol ($1\text{ }\mu\text{M}$)	NS 39.81 ± 2.97	NS 112.19 ± 4.72
Isoprenaline	Phentolamine ($10\text{ }\mu\text{M}$)	NS 41.21 ± 0.78	NS 120.16 ± 8.65
Isoprenaline	Spiroxatrine ($10\text{ }\mu\text{M}$)	NS 40.35 ± 0.90	NS 117.76 ± 9.22
Propranolol ($1\text{ }\mu\text{M}$)		1.61 ± 0.63	2.16 ± 0.34
Prazosin ($10\text{ }\mu\text{M}$)		0.98 ± 0.37	1.73 ± 0.25
Yohimbine ($10\text{ }\mu\text{M}$)		2.35 ± 1.01	3.55 ± 0.92
Phentolamine ($10\text{ }\mu\text{M}$)		3.26 ± 1.64	1.39 ± 0.51
Spiroxatrine ($10\text{ }\mu\text{M}$)		4.18 ± 2.83	2.85 ± 1.12
Haloperidol ($1\text{ }\mu\text{M}$)		0.68 ± 0.29	1.76 ± 0.91
Spiroperidol ($1\text{ }\mu\text{M}$)		1.22 ± 1.94	1.06 ± 0.61
(+)-Butaclamol ($1\text{ }\mu\text{M}$)		1.42 ± 0.86	1.33 ± 1.65
(-)-Butaclamol ($1\text{ }\mu\text{M}$)		0.78 ± 0.45	0.06 ± 1.14

None of the antagonists used stimulated glycogenolysis or cyclic AMP production. Results are expressed in terms of the % of $[^3\text{H}]$ -glycogen hydrolysis relative to control and % stimulation of cyclic AMP production above basal levels, and are given as means $\pm$ s.e.mean where n is from 8–12 separate experiments. NA = noradrenaline. * and NS denotes significantly and insignificantly different from agonist-induced response alone.

Table 3 Effects of various antagonists on 5-hydroxytryptamine (5-HT)/8-OH-DPAT induced [3 H]-glycogen hydrolysis or cyclic AMP production

<i>Agent</i>	<i>Antagonist added</i>	<i>% [^{3}H]-glycogen hydrolysis</i>	<i>% cyclic AMP production</i>
Buffer		0	0
5-HT (500 μ M)		38.90 $\pm$ 1.46	139.02 $\pm$ 7.36
5-HT	Spiroxitrine (1 μ M)	*6.70 $\pm$ 0.77	*25.42 $\pm$ 9.58
5-HT	Spiroxitrine (10 μ M)	*3.28 $\pm$ 0.42	*24.24 $\pm$ 8.08
5-HT	Ketanserin (10 μ M)	NS 36.59 $\pm$ 1.45	NS 130.01 $\pm$ 9.14
5-HT	Mianserin (10 μ M)	NS 39.44 $\pm$ 1.80	NS 136.85 $\pm$ 10.05
5-HT	Methysergide (10 μ M)	NS 37.18 $\pm$ 0.66	NS 132.04 $\pm$ 13.64
5-HT	Propranolol (10 μ M)	NS 37.27 $\pm$ 0.94	NS 132.58 $\pm$ 3.37
5-HT	MDL-72222 (10 μ M)	NS 38.99 $\pm$ 1.87	NS 141.35 $\pm$ 12.90
5-HT	Haloperidol (10 μ M)	NS 39.27 $\pm$ 0.53	NS 134.97 $\pm$ 5.82
8-OH-DPAT (500 μ M)		33.06 $\pm$ 1.82	128.86 $\pm$ 10.07
8-OH-DPAT	Spiroxitrine (1 μ M)	*5.08 $\pm$ 1.19	*16.88 $\pm$ 6.20
8-OH-DPAT	Spiroxitrine (10 μ M)	*2.18 $\pm$ 0.73	*9.02 $\pm$ 2.12
8-OH-DPAT	Methysergide (10 μ M)	NS 32.23 $\pm$ 3.02	NS 115.04 $\pm$ 7.30
8-OH-DPAT	MDL-72222 (10 μ M)	NS 33.70 $\pm$ 4.60	NS 130.31 $\pm$ 12.84
8-OH-DPAT	Propranolol (10 μ M)	NS 32.45 $\pm$ 3.48	NS 124.93 $\pm$ 11.73
8-OH-DPAT	Haloperidol (10 μ M)	NS 34.01 $\pm$ 2.75	NS 126.33 $\pm$ 8.17
RU24696 (500 μ M)		31.93 $\pm$ 1.61	104.85 $\pm$ 7.98
RU24696	Spiroxitrine (1 μ M)	*4.18 $\pm$ 0.21	*10.74 $\pm$ 0.96
RU24696	Spiroxitrine (10 μ M)	*1.83 $\pm$ 0.96	*4.60 $\pm$ 0.48
RU24696	Methysergide (10 μ M)	NS 31.59 $\pm$ 2.23	NS 107.28 $\pm$ 8.62
RU24696	MDL-72222 (10 μ M)	NS 32.26 $\pm$ 1.30	NS 106.79 $\pm$ 6.93
RU24696	Haloperidol (10 μ M)	NS 31.51 $\pm$ 0.91	NS 101.98 $\pm$ 2.19
Spiroxitrine (1 μ M)		2.12 $\pm$ 1.36	-1.21 $\pm$ 3.67
Ketanserin (10 μ M)		0.43 $\pm$ 0.65	1.42 $\pm$ 0.77
Mianserin (10 μ M)		1.87 $\pm$ 1.45	4.19 $\pm$ 2.08
Methysergide (10 μ M)		2.70 $\pm$ 1.55	0.74 $\pm$ 1.72
MDL-72222 (10 μ M)		0.79 $\pm$ 0.37	2.63 $\pm$ 1.05
Haloperidol (10 μ M)		1.90 $\pm$ 1.75	-0.19 $\pm$ 1.66

None of the antagonists used stimulated glycogen hydrolysis or cyclic AMP production. Results are expressed in terms of the % of [3 H]-glycogen hydrolysis relative to control and % stimulation of cyclic AMP production above basal levels, and are given as means $\pm$ s.e.mean. *n* is from 10–14 separate experiments, except for RU24696 results where *n* = 3. 8-OH-DPAT = 8-hydroxy-dipropylaminetetralin. * and NS denote significantly and insignificantly different from agonist-induced response alone.

retina while dopamine receptors were not significantly involved in glycogenolysis.

Ketanserin (10 μ M), mianserin (10 μ M) and methysergide (10 μ M), all 5-HT₂ antagonists, did not influence [3 H]-glycogen hydrolysis induced by either 5-HT or 8-OH-DPAT (Table 3). Furthermore, MDL-72222 (10 μ M), a peripheral 5-HT₃ receptor antagonist (Fozard, 1984), propranolol and haloperidol (10 μ M) were without effect (Table 3). In contrast, spiroxitrine (1 μ M), a 5-HT_{1A} antagonist was a potent blocker of the 5-HT- and 8-OH-DPAT-induced responses (Table 3). To characterize further the 5-HT-induced response, RU24696 (a 5-HT_{1A/B} agonist) was examined. This agonist stimulated [3 H]-glycogen hydrolysis to an extent similar to 8-OH-DPAT and the response was inhibited by spi-

roxatrine while other receptor antagonists had no effect (Table 3). Since 8-OH-DPAT is a 5-HT_{1A} receptor agonist (Middlemiss & Fozard, 1983; Hamon *et al.*, 1984; Hoyer *et al.*, 1986), it is concluded that 5-HT and 8-OH-DPAT promote glycogenolysis in the retina most probably via 5-HT_{1A} receptor sites.

The effect of VIP on the hydrolysis of [3 H]-glycogen in rabbit retinal slices also appears to be mediated through specific receptors. Propranolol (10 μ M), yohimbine (10 μ M), spiroxitrine (10 μ M), haloperidol (10 μ M) and methysergide (10 μ M) were all without effect upon the VIP-induced stimulation of glycogenolysis. Thus the VIP-induced response appeared not to be mediated by adrenoceptors, 5-HT or dopamine receptors.

The involvement of cyclic AMP in mediating glycogenolysis is demonstrated in Tables 1, 2 and 3. Except for dopamine, only substances which stimulated cyclic AMP production in retinal homogenates promoted glycogenolysis in rabbit retinal slices (Table 1). Additionally, db cyclic AMP (250 μM), a synthetic analogue of cyclic AMP which crosses the cell membrane (Posternak *et al.*, 1962), induced a more than 62% increase in glycogen breakdown in retinal slices, and neuronal and Müller cell cultures. Interestingly CCh induced a slight decrease in cyclic AMP production but had no effect on [^3H]-glycogen levels. Tables 2 and 3 show the influence of various antagonists upon the stimulation of cyclic AMP production but had no effect on [^3H]-glycogen levels. Tables 2 and 3 show the influence of various antagonists upon the stimulation of cyclic AMP production caused by different agonists. These results suggest that in the rabbit retina, NA/adrenaline, 5-HT and VIP increase cyclic AMP production and induce an enhanced rate of glycogen breakdown through the same receptor types.

Retinal cultures

Rabbit retinal cultures at 3–5 and 25–30 days old were used in an effort to determine the cellular site of the agonist-induced [^3H]-glycogen hydrolysis. This is possible since 3–5 day old cultures contain both neurones and Müller cells, whereas only non-neuronal retinal (Müller) cells are found in the 25–30 days cultures (Osborne, 1987; Osborne *et al.*, 1988; Ghazi & Osborne, 1988b). In both young and old

cultures, NA, isoprenaline, VIP, 5-HT and 8-OH-DPAT stimulated the hydrolysis of [^3H]-glycogen to an extent similar to that found in intact retinal slices (Tables 4 and 5). Furthermore, pharmacological characterization of this response in both culture types proved to be identical to that found in retinal slices. Tables 4 and 5 demonstrate to what extent the various agents stimulated the production of cyclic AMP in 3–5 and 25–30 day old cultures. It is evident that these cyclic AMP responses are fairly similar to those shown in Table 1 for retinal homogenates, with two exceptions: (1) isoprenaline (500 μM) induced a significantly higher stimulation of cyclic AMP production in both culture types than in retinal homogenates and (2) dopamine (500 μM) and apomorphine (500 μM) were only able to elicit a cyclic AMP response in young 3–5 day old retinal cultures. It appears that the adenylate cyclase-linked dopamine receptors are only located on neurones, as no response was found in the Müller cell cultures (25–30 days). The results obtained in the 25–30 day old cultures suggest the presence of functional muscarinic receptor, β -adrenoceptor, 5-HT_{1A} and VIP receptors, linked to cyclic AMP production, on Müller cells.

Discussion

NA, isoprenaline, VIP, 5-HT and 8-OH-DPAT induced [^3H]-glycogen hydrolysis in rabbit retinal slices and retinal cultures of different ages. The NA/

Table 4 Percentage stimulation of [^3H]-glycogen hydrolysis (glycogenolysis) or cyclic AMP production over basal levels in 3–5 day old rabbit retinal cultures by various agents

Agent	Conc (μM)	% [^3H]-glycogen hydrolysis	% cyclic AMP production
Buffer	—	0	0
NA	500	52.41 $\pm$ 1.85	164.23 $\pm$ 4.74
Isoprenaline	500	50.32 $\pm$ 5.42	235.59 $\pm$ 14.40
VIP	5	54.72 $\pm$ 2.14	393.23 $\pm$ 29.89
5-HT	500	37.77 $\pm$ 1.91	107.14 $\pm$ 5.57
8-OH-DPAT	500	33.05 $\pm$ 2.20	90.17 $\pm$ 4.04
GABA	500	NS 4.03 $\pm$ 0.58	NS 5.88 $\pm$ 1.23
Glycine	500	NS 3.73 $\pm$ 0.73	NS 4.45 $\pm$ 3.22
Taurine	500	NS 1.78 $\pm$ 0.98	NS 1.82 $\pm$ 1.52
Dopamine	500	NS 8.28 $\pm$ 1.53	143.77 $\pm$ 6.16
Apomorphine	500	NS 2.45 $\pm$ 0.79	165.92 $\pm$ 3.20
Carbachol	500	NS 8.59 $\pm$ 2.96	—35.40 $\pm$ 3.80
db cyclic AMP	250	68.79 $\pm$ 1.74	

Results are given as means $\pm$ s.e.mean where *n* is from 6–14 separate experiments. NA = noradrenaline, VIP = vasoactive intestinal peptide, 8-OH-DPAT = 8-hydroxy-dipropylaminetetralin, GABA = γ -aminobutyric acid and db cyclic AMP = dibutyl cyclic AMP. NS denotes insignificant compared with controls.

Table 5 Percentage stimulation of [^3H]-glycogen hydrolysis (glycogenolysis) or cyclic AMP production over basal levels in 25–30 days old rabbit retinal cultures (primarily Müller cells) by various agents

Agent	Conc (μM)	% [^3H]-glycogen hydrolysis	% cyclic AMP production
Buffer	—	0	0
NA	500	50.38 $\pm$ 1.61	186.72 $\pm$ 3.89
Isoprenaline	500	49.47 $\pm$ 1.68	223.09 $\pm$ 26.33
VIP	5	49.18 $\pm$ 2.19	392.02 $\pm$ 39.38
5-HT	500	35.68 $\pm$ 1.26	88.00 $\pm$ 5.58
8-OH-DPAT	500	32.96 $\pm$ 1.86	66.99 $\pm$ 6.01
GABA	500	NS 6.00 $\pm$ 0.99	NS 4.62 $\pm$ 1.19
Glycine	500	NS 6.60 $\pm$ 0.96	NS 4.15 $\pm$ 1.36
Taurine	500	NS 3.34 $\pm$ 1.42	NS 2.90 $\pm$ 0.86
Dopamine	500	NS 3.90 $\pm$ 1.33	NS 6.74 $\pm$ 3.65
Apomorphine	500	NS 4.25 $\pm$ 1.24	NS 3.33 $\pm$ 0.88
Carbachol	500	NS 4.28 $\pm$ 0.58	— 30.38 $\pm$ 6.75
db cyclic AMP	250	69.28 $\pm$ 1.76	

Values are means $\pm$ s.e.mean where n is from 6–14 separate experiments. NA = noradrenaline, VIP = vasoactive intestinal peptide, 8-OH-DPAT = 8-hydroxy-dipropylaminetetralin, GABA = γ -aminobutyric acid, and db cyclic AMP = dibutyryl cyclic AMP. NS denotes insignificant compared with controls.

isoprenaline and 5-HT/8-OH-DPAT responses appear to be mediated via β -adrenoceptors and 5-HT₁ receptors (possibly 5-HT_{1A}), respectively, while the VIP-induced response is mediated through another receptor subtype. The results obtained in the non-neuronal (Müller) cell cultures establish the presence of functional receptors on these cells. With the exception of dopamine and apomorphine, agonists which stimulated the formation of cyclic AMP also promoted glycogenolysis. Agonist-induced stimulation of glycogenolysis and cyclic AMP formation were blocked to a similar extent by the same antagonist and both responses are probably mediated through the same receptor subtypes. Furthermore, db cyclic AMP strongly induced glycogenolysis in both retinal slices and cultured retinal cells. It seems probable that agonist-induced [^3H]-glycogen hydrolysis correlates with stimulated production of cyclic AMP.

The effect of noradrenaline on glycogenolysis in the retina resembles observations made elsewhere. NA is known to decrease newly synthesized intracellular glycogen in mouse cortical slices (Quach *et al.*, 1978; Magistretti *et al.*, 1981) and in cultured astroglia (Magistretti *et al.*, 1983) by at least 60%. In astrocytoma cells, cultured and transformed astrocytes, NA and isoprenaline significantly increased the breakdown of glycogen (Passonneau & Crites, 1976; Cummins *et al.*, 1983a,b). Isoprenaline was also found to induce glycogenolysis in rat striatal slices (Wilkening & Makman, 1976; 1977) and in chick cerebral hemispheres (Edwards *et al.*, 1974). NA- and isoprenaline-mediated effects were through

β -adrenoceptors. Interestingly, haloperidol partially blocked the NA-induced stimulation of cyclic AMP production and glycogen breakdown in intact retina and 3–5 day retinal cultures but not in the older Müller cell cultures. Whilst haloperidol is a classical dopamine antagonist it would appear that this effect is elicited via adrenoceptors rather than dopamine receptors. Other dopamine receptor antagonists, viz spiroperidol and butaclamol, did not nullify the NA-induced responses. Furthermore neither dopamine nor apomorphine stimulated glycogenolysis. Since both dopamine and apomorphine strongly stimulated cyclic AMP production without influencing glycogen breakdown, it is clear that not all receptors linked to cyclic AMP production promote glycogenolysis. Interestingly, an inactive form of cyclic AMP-dependent protein kinase containing bound cyclic AMP has been purified and characterized (Cobb *et al.*, 1987). This may help explain the inability of either dopamine or apomorphine to stimulate glycogen breakdown.

As is the case for mouse cortical slices (Quach *et al.*, 1978), leech segmental ganglia (Seal & Pentreath, 1985) and cultured brain astroglia (Magistretti *et al.*, 1983), 5-HT induced a significant hydrolysis of [^3H]-glycogen in the various retinal preparations. 8-OH-DPAT and RU24696, known as 5-HT_{1A} receptor agonists (Middlemiss & Fozard, 1983; Hamon *et al.*, 1984; Sills *et al.*, 1984), mimicked the effect produced by 5-HT and were likewise strongly inhibited by the 5-HT_{1A} receptor antagonist, spiroxatrine. Furthermore the dose-response curves and EC₅₀ values for both 5-HT- and 8-OH-DPAT-induced effects on

[^3H]-glycogen hydrolysis were very similar. Thus it appears that 5-HT induces glycogenolysis in the retina via 5-HT $_{1A}$ receptors.

VIP was the most potent of all the agents tested in inducing glycogenolysis in the different retinal preparations. VIP has a similar potent effect on glycogenolysis in mouse cortical slices, cultured rat astroglia (Magistretti *et al.*, 1981; 1983), hepatocytes (Wood & Blum, 1982), cultured chick retinal cells (Koh *et al.*, 1984) and a human colon cell line (Rousset *et al.*, 1981) and vertebrate retina (Schorderet *et al.*, 1984). The glycogenolytic effect of VIP in retinal tissue and cell culture was not inhibited by any of the antagonists tested, including those that blocked the response induced by either NA or 5-HT. These results, particularly from the culture studies, show that the VIP response is not mediated indirectly by the release of either NA or 5-HT from other cells but rather point to an involvement of a specific VIP receptor type in mediating [^3H]-glycogen breakdown.

The results described in this study suggest that agonist-induced glycogen hydrolysis involves the generation of cyclic AMP. However, other factors may contribute to the glycogenolytic effects of the different agonists. For example, in the CNS, as in other tissues, phosphorylase *b* kinase is activated by agonists which mediated a Ca^{2+} influx (this issue is discussed below) (Cohen, 1982; Ververken *et al.*, 1982). Additionally, Opler & Makman (1972) demonstrated that, in contrast to NA, histamine induces glycogenolysis in rat astrocytoma cells without activating adenylate cyclase activity. These authors also concluded from their studies that the histamine effect may be mediated by an elevation of cyclic AMP levels through an action on the phosphodiesterase activity (Opler & Makman, 1972). More recently, Pearce *et al.* (1985) have produced data to suggest that α -adrenoceptors, through a cyclic AMP-independent mechanism, stimulate glycogen turnover. In the present study no evidence was found for the involvement of α -adrenoceptors in either stimulating cyclic AMP production or promoting glycogenolysis. Moreover, CCh does not stimulate glycogen breakdown or cyclic AMP production. Furthermore, NA in the presence of propranolol can promote inositol phosphates (IPs) accumulation but has no effect on cyclic AMP formation or glycogen hydrolysis. These results argue against the involvement of the IPs/DG 2nd messenger system in mediating glycogenolytic events in retinal cells.

In the present study, only agents which stimulated cyclic AMP formation also promoted glycogen breakdown. Moreover, the EC_{50} values for NA, 5-HT and VIP are similar for both stimulation of cyclic AMP formation (unpublished results) and glycogenolysis, thus arguing in favour of the idea that

the same receptors are involved in the two responses and that similar receptor occupancies are necessary to elicit each of the effects. This is not the case in mouse cortical slices where a lower receptor occupancy is required for the stimulation of [^3H]-glycogen hydrolysis than for promoting cyclic AMP production (Quach *et al.*, 1978). The possibility that external Ca^{2+} might be involved in the agonist-induced stimulation of glycogenolysis in retinal tissues is unlikely, because similar results were obtained in Ca^{2+} -free medium for each of the agonists tested (unpublished results). Another possible mechanism is that agonists induce an activation of glycogen hydrolysis by mobilizing intracellular stores of Ca^{2+} . This is very unlikely since agents such as VIP, isoprenaline and 8-OH-DPAT do not induce a rise in free intracellular Ca^{2+} (Ghazi & Osborne, 1988b) but significantly stimulate glycogenolysis in retinal cells. Moreover, NA and 5-HT induce a rise in free intracellular Ca^{2+} levels that are respectively blocked by prazosin and ketanserin and these two antagonists have no effect on glycogenolysis (Ghazi & Osborne, 1988b).

Previous investigations in rat striatal slices and chick cerebral hemispheres also showed the involvement of cyclic AMP in the glycogenolytic responses induced by isoprenaline (Edwards *et al.*, 1974; Wilkening & Makman, 1976; 1977). In the present study the synthetic cyclic AMP analogue, db cyclic AMP, displayed a very potent glycogenolytic effect in rabbit retinal slices and retinal cultures as described before for rat astrocytoma cells (Opler & Makman, 1972). This suggests that the cyclic AMP-dependent phosphorylation cascade is one of the operative mechanisms in hydrolysing glycogen in retinal cells. This is supported by the finding that NA stimulated the conversion of phosphorylase *b* into its more active form phosphorylase *a* which hydrolyses the glycogen chain through increasing cyclic AMP (Edwards *et al.*, 1974). Furthermore an increase in the amount of phosphorylase *a* in rat astrocytoma and neuroblastoma cells has been reported following treatment with a phosphodiesterase inhibitor (Passonneau & Crites, 1976).

The primary site for glycogen storage and synthesis in the rabbit retina is the inner portion of the Müller cells (Kuwabara & Cogan, 1961; Magalhaes & Coimbra, 1970; 1972). Nearly all glucose-6-phosphatase activity, which mainly hydrolyses glucose-6-phosphate that emanates from glycogen breakdown, has been localised in the inner portion of the Müller cells (Magalhaes & Coimbra, 1972). These observations suggest that Müller cells are the main storage area of glycogen and glucose formation; retinal neurones and photoreceptors possess negligible glycogen stores. Since systemic glucose and oxygen are not directly supplied to the inner layers of the rabbit

retina, the glucose formed and released from the Müller cells could be utilized in anaerobic glycolysis in the deprived parts of the retina. Additionally, Koh *et al.* (1984) have demonstrated that VIP and glucagon stimulate glycogen breakdown in cultured Müller cells from the avian retina. Thus the present results could be interpreted as suggesting that substances like NA, VIP and 5-HT are released directly

by retinal neurones following a physiological event or alternatively transported as hormones to activate glycogenolysis in the Müller cells. This results in the formation of a supply of glucose for ultimate utilization by surrounding neurones.

This work was supported by a grant from the Wellcome Trust and the Nuffield Foundation.

References

- BEALE, R., NICHOLAS, D., NEUHOFF, V. & OSBORNE, N.N. (1982). The binding of tetanus toxin to retinal cells. *Brain Res.*, **248**, 141–149.
- BRADFORD, M.M. (1976). A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. *Analyt. Biochem.*, **72**, 248–254.
- BROWN, B.L. & MAKMAN, M.H. (1972). Stimulation by dopamine of adenylate cyclase in retinal homogenates and of adenosine 3',5'-cyclic monophosphate formation in intact Retina. *Proc. Natl. Acad. Sci. U.S.A.*, **69**, 539–543.
- COBB, C.E., BETH, A.H. & CORBIN, J.D. (1987). Purification and characterization of an inactive form of cAMP-dependent protein kinase containing bound cAMP. *J. Biol. Chem.*, **262**, 16566–16574.
- COHEN, P. (1982). The role of protein phosphorylation in neural and hormonal control of cellular activity. *Nature*, **296**, 613–620.
- CUMMINS, C.J., LUST, W.D. & PASSONNEAU, J.V. (1983a). Regulation of glycogen metabolism in primary and transformed astrocytes *in vitro*. *J. Neurochem.*, **40**, 128–136.
- CUMMINS, C.J., LUST, W.D. & PASSONNEAU, J.V. (1983b). Regulation of glycogenolysis in transformed astrocytes *in vitro*. *J. Neurochem.*, **40**, 137–144.
- EDWARDS, C., NAHORSKI, S.R. & ROGERS, K.J. (1974). *In vivo* changes of cerebral cyclic adenosine 3',5'-monophosphate induced by biogenic amines: association with phosphorylase activation. *J. Neurochem.*, **22**, 565–572.
- FOZARD, J.R. (1984). MDL 72222 a potent and highly selective antagonist at neuronal 5-hydroxytryptamine receptors. *Naunyn-Schmiedeberg's Arch. Pharmacol.*, **326**, 36–44.
- GHAZI, H. & OSBORNE, N.N. (1988a). Agonist-induced stimulation of inositol phosphates in primary retinal cultures. *J. Neurochem.*, **50**, 1851–1858.
- GHAZI, H. & OSBORNE, N.N. (1988b). Receptors linked to phosphoinositide-specific PLC increase free intracellular calcium in rabbit retinal cultures. *Neurochem. Int.*, (in press).
- GÖTHERT, M. & SCHLICKE, E. (1987). Classification of serotonin receptors. *J. Cardiovasc. Pharmacol.*, **10**, S3–S7.
- HAMON, M., BOURGOIN, S., GOZLAN, H., HALL, M.D., GOETZ, C., ARTAND, F. & HORN, A.S. (1984). Biochemical evidence for the 5-HT agonist properties of PAT (8-hydroxy-2-(di-n-propylamino)tetralin) in the rat brain. *Eur. J. Pharmacol.*, **100**, 263–276.
- HOYER, D., PAZOS, A., PROBST, A. & PALACIOS, J.M. (1986). Serotonin receptors in the human brain: I. Characterization and autoradiographic localization of 5-HT_{1A} recognition sites. Apparent absence of 5-HT_{1B} recognition sites. *Brain Res.*, **376**, 85–96.
- KEBABIAN, J.W., PETZOLD, G.L. & GREENGARD, P. (1972). Dopamine-sensitive adenylate cyclase in caudate nucleus of rat brain, and its similarity to the "dopamine receptor". *Proc. Natl. Acad. Sci. U.S.A.*, **69**, 2145–2149.
- KOH, S-W.M., KYRITSIS, A. & CHADER, G.J. (1984). Interaction of neuropeptides and cultured glial (Müller) cells of the chick retina: elevation of intracellular cyclic AMP by vasoactive intestinal peptide and glucagon. *J. Neurochem.*, **43**, 199–203.
- KUWABARA, T. & COGAN, D. (1961). Retinal glycogen. *Arch. Ophthalmol.*, **66**, 680–688.
- MAGALHAES, M.M. & COIMBRA, A. (1970). Electron microscope radioautographic study of glycogen synthesis in the rabbit retina. *J. Cell. Biol.*, **47**, 263–275.
- MAGALHAES, M.M. & COIMBRA, A. (1972). The rabbit retina Müller cell. A fine structural and cytochemical study. *J. Ultrastruct. Res.*, **39**, 310–326.
- MAGISTRETTI, P.J., MORRISON, J.H., SHOEMAKER, W.J., SAPIN, V. & BLOOM, F.E. (1981). Vasoactive intestinal polypeptide induces glycogenolysis in mouse cortical slices: a possible regulatory mechanism for the local control of energy metabolism. *Proc. Natl. Acad. Sci. U.S.A.*, **78**, 6525–6539.
- MAGISTRETTI, P.J., MANTHORPE, M., BLOOM, F.E. & VARON, S. (1983). Functional receptors for vasoactive intestinal polypeptide in cultured astroglia from neonatal rat brain. *Regulat. Peptides*, **6**, 71–80.
- MAGISTRETTI, P.J., MORRISON, J.H., SHOEMAKER, W.J. & BLOOM, F.E. (1984). Morphological and functional correlates of VIP neurons in cerebral cortex. *Peptides*, **5**, 213–218.
- MAGISTRETTI, P.J., HOF, P.R. & MARTIN, J.-L. (1986). Adenosine stimulates glycogenolysis in mouse cerebral cortex: A possible coupling mechanism between neuronal activity and energy metabolism. *J. Neurosci.*, **6**, 2558–2562.
- MIDDLEMISS, D.N. & FOZARD, J.R. (1983). 8-hydroxy-2-(di-n-propylamino)-tetralin discriminates between subtypes of the 5-HT₁ recognition site. *Eur. J. Pharmacol.*, **90**, 151–153.
- MIZUNO, K. & SATO, K. (1975). Reassessment of histochemistry of retinal glycogen. *Exp. Eye Res.*, **21**, 489–497.
- NAHORSKI, S.R. & ROGERS, K.J. (1972). An enzymic fluorometric micro-method for determination of glycogen. *Analyt. Biochem.*, **49**, 492–497.
- NELSON, D.L. & TAYLOR, E.W. (1986). Spiroaxatine: a selec-

- tive serotonin_{1A} receptor antagonist. *Eur. J. Pharmacol.*, **124**, 207–208.
- OPLER, L.A. & MAKMAN, M.H. (1972). Mediation by cyclic AMP of hormone-stimulated glycogenolysis in cultured rat astrocytoma. *Biochem. Biophys. Res. Commun.*, **46**, 1140–1145.
- OSBORNE, N. (1987). Neurone-glia relationship in the retina: localization of GABA uptake and endogenous GFAP and GABA immunoreactivities. In *Glial-Neuronal Communication in Development and Regeneration*. ed. Althans, H.H. & Seifert, W. pp. 117–129. Heidelberg: Springer-Verlag.
- OSBORNE, N. & BEATON, D.W. (1986). Studies on serotonin-accumulating cells in rabbit retinal cultures. *Brain Res.*, **362**, 287–298.
- OSBORNE, N.N., MORGAN, B., GHAZI, H. & BEATON, D. (1988). Correlation between vimentin, GABA and GFAP immunoreactivities and ³H-GABA and ³H-Taurine uptake in intact retina and retinal cell cultures. *Biogenic Amines*, **5**, 249–268.
- PASSONNEAU, J.V. & CRITES, S.K. (1976). Regulation of glycogen metabolism in astrocytoma and neuroblastoma cells in culture. *J. Biol. Chem.*, **251**, 2015–2022.
- PASSONNEAU, J.V. & LAUDERDALE, V.R. (1974). A comparison of three methods of glycogen measurement in tissues. *Analyt. Biochem.*, **60**, 405–412.
- PEARCE, B.R., CAMBRAY-DEAKIN, M. & MURPHY, S. (1985). Glial glycogen stores are regulated by α -adrenergic receptors. *Biochem. Soc. Trans.*, **13**, 232–233.
- POSTERNAK, T., SUTHERLAND, E.W. & HENION, W.F. (1962). Derivatives of cyclic 3',5'-adenosine monophosphate. *Biochem. Biophys. Acta.*, **65**, 558–560.
- QUACH, T.T., ROSE, C. & SCHWARTZ, J.C. (1978). [³H]Glycogen hydrolysis in brain slices: Responses to neurotransmitters and modulation of noradrenaline receptors. *J. Neurochem.*, **30**, 1335–1341.
- QUACH, T.T., DUCHEMIN, A.M., ROSE, C. & SCHWARTZ, J.C. (1980). ³H-Glycogen hydrolysis elicited by histamine in mouse brain slices: selective involvement of H₁-receptors. *Mol. Pharmacol.*, **1**, 301–308.
- QUACH, T.T., ROSE, C., DUCHEMIN, A.M. & SCHWARTZ, J.C. (1982). Glycogenolysis induced by serotonin in brain: identification of a new class of receptor. *Nature*, **298**, 373–375.
- ROUSSET, M., LABURTHER, M., CHEVALIER, G., BOISSARD, C., ROSSELIN, G. & ZWEIBAUM, A. (1981). Vasoactive intestinal peptide (VIP) control of glycogenolysis in the human colon carcinoma cell line HT-29 in culture. *FEBS Lett.*, **126**, 38–40.
- SCHORDERET, M., HOF, P. & MAGISTRETTI, P.J. (1984). The effects of VIP on cyclic AMP and glycogen levels in vertebrate retina. *Peptides*, **5**, 295–298.
- SEAL, L.H. & PENTREATH, V.W. (1985). Modulation of glial glycogen metabolism by 5-hydroxytryptamine in leech segmental ganglia. *Neurochem. Int.*, **7**, 1037–1045.
- SILLS, M.A., WOLFE, B.B. & FRAZER, A. (1984). Determination of selective and nonselective compounds for 5HT_{1A} and 5HT_{1B} receptor subtypes in rat frontal cortex. *J. Pharmacol. Exp. Ther.*, **231**, 480–487.
- SOLLING, H. & ESMANN, V. (1975). A sensitive method of glycogen determination in the presence of interfering substances utilizing the filter-paper technique. *Analyt. Biochem.*, **68**, 664–668.
- VERVERKEN, D., VAN VELDHoven, P., PROOST, C., CARTON, H. & DE WULF, H. (1982). On the role of calcium ions in the regulation of glycogenolysis in mouse brain cortical slices. *J. Neurochem.*, **38**, 1286–1295.
- WILKENING, D. & MAKMAN, M.H. (1976). Stimulation of glycogenolysis in rat caudate nucleus slices in 1-isopropyl norepinephrine, dibutyl cyclic AMP and 2-chloroadenosine. *J. Neurochem.*, **26**, 923–928.
- WILKENING, D. & MAKMAN, M.H. (1977). Activation of glycogen phosphorylase in rat caudate nucleus slices by 1-isopropyl norepinephrine and dibutyl cyclic AMP. *J. Neurochem.*, **28**, 1001–1007.
- WOOD, C.L. & BLUM, J.J. (1982). Effect of vasoactive intestinal polypeptide on glycogen metabolism in rat hepatocytes. *Am. J. Physiol.*, **242**, E262–E272.

(Received June 13, 1988

Revised November 30, 1988

Accepted December 7, 1988)