fertilization. To detect particle-associated promutagens, which require metabolic conversion to their mutagenic form, the S-9 microsomal fraction of liver was included (*4*). Significantly increased mortality and reduced vigor among autogamous progeny from treated parent cells were used as measures of treatment-induced damage to DNA.

Fly ash and heated fly ash were both significantly mutagenic ($P < .05$) when uninduced S-9 was added, reflecting the direct action of particle-associated mutagens (Table 1). Addition of induced S-9 increased the mutagenicity of unheated fly ash but not heated fly ash, indicating heat inactivation of indirect-acting mutagens associated with the particles. Fly ash and heated fly ash were optimally mutagenic at 535 and 1060 μg/ml, respectively. Additional evidence of mutagenicity was the appearance of morphological abnormalities including boomerang-shaped cells, bent bodies, and thin or bloated cells. Temperature-sensitive cells were also detected, predominantly among the survivors of clones that died at room temperature.

To further evaluate the nature of the heat-stable agents, the heated particles were extracted with hydrochloric acid or dimethyl sulfoxide (DMSO) before the cells were incubated with them (*19*). The HCl-extracted particles had no significant mutagenic effects, whereas the DMSO-extracted particles retained their mutagenicity (Table 2). This demonstrates that a heat-stable, aqueous acid-extractable mutagen is associated with fly ash. The solubility behavior and temperature stability are also consistent with the presence of inorganic mutagens.

Variations in the response of members of the same clone to fly ash–associated mutagens may reflect differences in susceptibility to damage. Possible causes for this include (i) differences in the threshold concentration of mutagenic particles ingested, (ii) variation in the clones' repair capability, and (iii) metabolism of the mutagenic agents to a harmless form.

Both the Ames *Salmonella* system and the *Paramecium* assay disclosed direct- and indirect-acting fly ash–associated mutagens (*2*, *3*). A major difference was the retention of mutagenic activity after heat alteration of fly ash in the protozoan assay only. Also, aqueous acid extraction did not alter mutagenesis in the Ames assay (*5*), but resulted in decreased mutagenicity in the protozoan system. This suggests protozoan sensitivity to inorganic mutagens. Although Fisher *et al.* (*5*) did not ascribe a significant component of fly ash mutagenicity to inorganic compounds, hexavalent chromium compounds, tri- and pentavalent arsenic, selenium, and *cis*-diamminedichloroplatinum II are mutagens in a variety of bacterial systems (*20*). Mammalian cell mutagenicity (or transformation) has been observed with these inorganic compounds as well as with soluble salts of cadmium, nickel, and beryllium. Thus, differences in the biological properties of fly ash in the two bioassays may reflect quantitative or qualitative differences in sensitivity.

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2-Amino-4-Phosphonobutyric Acid: A New Pharmacological Tool for Retina Research

Abstract. *Information processing in the vertebrate retina occurs in two separate channels known as* ON *and* OFF *channels. When intracellular electrophysiological recordings were obtained from the perfused retina-eyecup preparation of the mudpuppy (*Necturus maculosus*), the addition of 2-amino-4-phosphonobutyric acid to the bathing medium blocked all responses in the* ON *channel but left intact the* OFF *responses including* OFF *ganglion cell discharge. 2-Amino-4-phosphonobutyric acid blocks the light response of the* ON *bipolar cell by mimicking the endogenous photoreceptor transmitter.*

A characteristic feature of the vertebrate retina is the segregation of information processing into two separate channels. Focal light stimulation defines an ON channel that provides excitatory input to one class of ganglion cells at the onset of a light stimulus, while an OFF pathway provides excitatory input to a separate group of ganglion cells at the termination of a light flash (*1*). Both ON and OFF channels can be distinguished through many subcortical and early cortical neuronal interactions (*2*). These channels are separated at the first level of synaptic interaction where photoreceptor activity gives rise to two different types of bipolar cell responses, as shown by the polarity of the membrane potential change evoked by a focal light stimulus (*3*). Depolarizing bipolar cells provide the ON pathways, whereas hyperpolarizing bipolar cells underlie the OFF

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channel (*4*, *5*). Pharmacological techniques and ion replacement studies indicate that the activities of the ON and OFF channels are separated, suggesting that the two bipolar cell types may have different pharmacological properties (*6*) and ionic dependencies (*7*). However, it has not been possible to separate the two channels without altering other neurons as well. We report here the highly selective effects of DL-2-amino-4-phosphonobutyric acid (APB), an agent that in micromolar concentrations selectively blocks the ON channel while leaving unaffected the OFF channel and other neurons or neuronal response components that do not depend on the depolarizing bipolar cell input. The highly selective nature of the blocking action suggests that the synaptic receptor-ionophore complex associated with the ON bipolar cells is unique among the synaptic receptor population of retinal neurons. APB should prove useful in retina research for deciphering neuronal activity that is dependent on ON or OFF pathways, the nature of the synaptic receptor of the ON bipolar cells, and the structure of the endogenous transmitter whose action by our analysis is mimicked by APB.

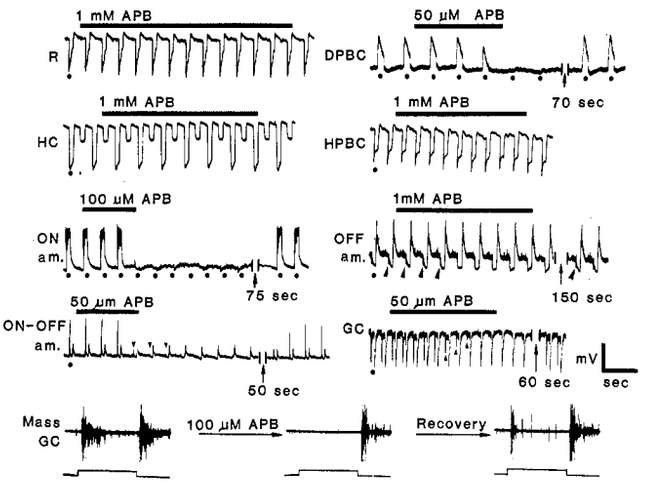

Fig. 1. Effects of APB on intracellularly recorded responses of a receptor cell (*R*), ON bipolar cell (*DPBC*), horizontal cell (*HC*), OFF bipolar cell (*HPBC*), three types of amacrine cells (*ON am.*, *OFF am.*, and *ON-OFF am.*), a ganglion cell (*GC*), and extracellularly recorded mass ganglion cell discharge (*Mass GC*). The filled circle or raised line under each trace indicates a 2-second, diffuse light stimulus (irradiance = 3×10^{-8} watt/cm^2) which is repeated every 10 seconds. In horizontal cells, a 200-μm small spot (small circle) alternated with the diffuse light. The bar above each trace indicates the concentration and duration of APB application. With 50 to 100 μM APB, the light responses of the ON bipolar and amacrine cells and the ON components of the ON-OFF amacrine and ganglion cells were reversibly blocked, whereas with 1 mM APB, responses of the receptor cell and OFF bipolar cell and the OFF responses of the amacrine and ganglion cells were unaffected. The ON, but not the OFF, mass ganglion cell discharge was eliminated after a 2-minute exposure to APB; recovery was observed after 10 minutes. Calibration: 13 mV for *R*, *DPBC*, and *HPBC*; 20 mV for *HC*, *am.*, and *GC*. Time bar is 20 seconds. Arrows indicate progressive changes in the ON components due to APB. Positivity is an upward deflection in this and Fig. 2.

Intracellular recordings were obtained from all types of retinal neurons in the perfused retina-eyecup preparation of the mudpuppy. The eyecups were bathed in an amphibian Ringer solution, maintained at *p*H 7.8 with 5 mM Hepes [4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid] buffer and aerated with 100 percent O_2. The bathing medium could be switched from a control perfusate to one containing various pharmacological agents by switching stopcock controls for each solution. The delay in changing from one perfusate to another was short so that the effects from introducing pharmacological agents were evident within seconds [for details of this technique see (*4*)]. In some experiments, a brief current pulse of $\pm$ 0.1 nA was applied to the electrode and the bridge circuit of a WP 707 amplifier in order to evaluate changes in input resistance associated with the application of test agents.

Figure 1 shows photographic reproductions of intracellular recordings from the major cell types in the mudpuppy retina. The bottom row of traces (row 5) illustrates extracellular recordings of mass ganglion cell discharge obtained by stainless steel electrodes placed on the retinal surface, amplified by a Grass P15 amplifier, and photographed from a display on a storage oscilloscope (Tektronix 5100). In each intracellular trace (rows 1 to 4), an intermittent diffuse light stimulus, 2 seconds in duration, was presented once every 10 seconds; in some cells (horizontal cells) the diffuse light stimulus alternated with a 200-μm small spot flash centered over the recording electrode. Rows 1 and 2 show recordings from outer retinal neurons. Cells were identified by physiological criteria (*3*); photoreceptor identification was also aided by the application of 3 mM cobalt which rapidly blocked the light response of second- and third-order neurons, but had little effect on receptors, provided the exposure to cobalt was only a few minutes in duration (*8*).

High concentrations of APB (1 mM) did not affect the receptor cells, horizontal cells, or OFF bipolar cells, but a much smaller dose (50 μM) reversibly blocked the light response of ON bipolar cells. The blocking action of APB was not associated with any significant change in membrane potential, although in other cells (Fig. 2, top trace) a slight hyperpolarization was observed.

The traces in rows 3, 4, and 5 of Fig. 1 show the effects of APB on inner retinal neurons. The light-evoked response of an ON amacrine cell was rapidly and reversibly blocked by 100 μM APB, whereas the response of an OFF amacrine cell was minimally affected by 1 mM APB. A small depolarizing ON component of the light response of an OFF amacrine cell was blocked by APB, leaving a more sustained hyperpolarizing response at light onset. This small ON depolarization returned after the APB perfusate was switched to a control. The left-hand trace of row 4, Fig. 1, shows a recording from an ON-OFF amacrine cell in which there were transient depolarizing responses at the onset and termination of a light stimulus. A comparatively low dose (50 μM) of APB blocked the large ON response, while leaving a smaller OFF response. The OFF response that persisted in APB was the same amplitude as the control OFF response. Rapid recovery of the ON response is shown after a 50-second break in the continuous penwriter record. An ON-OFF ganglion cell recording is illustrated in the right-hand trace of row 4. When these cell types are depolarized by microelectrode penetration, impulse activity is commonly lost and the response is usually dominated by exaggerated inhibitory postsynaptic potentials (IPSP's), which, in the recording of Fig. 1, are the only obvious response components. The ON IPSP is rapidly blocked by 50 μM APB, whereas the OFF IPSP is comparatively unaffected. The traces in row 5 of Fig. 1 show that 100 μM APB selectively and reversibly blocks the ON mass ganglion cell discharge. In addition, 1 mM APB blocked the b wave of the electroretinogram while leaving the d wave and the P3 component. APB also appears to block selectively the action of ON responses in the perfused rabbit retina.

In our experiments the selectivity of APB was maintained for concentrations up to 1 mM, which is 20 times the satu-

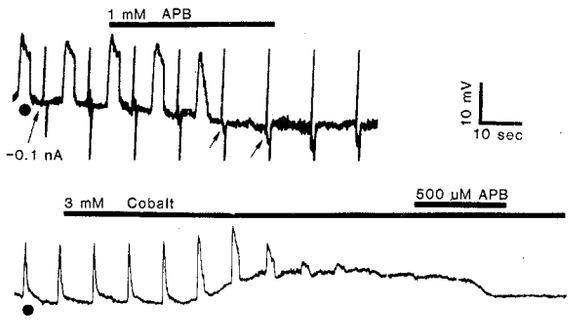

Fig. 2. Intracellular recordings from ON bipolar cells exposed to a 2-second, diffuse light stimulus once every 10 seconds (irradiance = 3×10^{-8} watt/cm^2). In the top trace, a 0.1-nA negative current pulse (arrow) was applied between light stimuli to monitor relative changes in input resistance. With 1 m*M* APB the light response was eliminated and the input resistance of the cell was increased. In the bottom trace, a second ON bipolar cell was exposed to 3 m*M* cobalt which depolarized the cell and blocked the light response. When 500 μM APB was superimposed on the cobalt the cell hyperpolarized. These observations suggest that APB is an agonist that mimics the action of the endogenous photoreceptor transmitter.

rating dose for blocking the ON responses, but at higher concentrations (20 m*M*) of APB we observed some blocking effect on the light response of horizontal cells.

The most parsimonious explanation of the action of APB is that it blocks the response of the ON bipolar cells and that the loss of ON responses in the inner retina reflects a loss of synaptic drive from the ON bipolar cells. There are three general mechanisms by which APB might block the light response of the ON bipolar cells. One possibility is that the agent blocks synaptic input to the bipolar cell. However, a block of synaptic drive is associated with the depolarization of ON bipolar cells (*8*, *9*) (see Fig. 2). A second mechanism is the elimination of the light response by decreasing the input resistance to effectively short-circuit the synaptic potentials. A decrease in input resistance could be a direct or synaptically mediated change. To test this possibility, we measured the relative change in input resistance associated with APB action by applying a brief − 0.1-nA current pulse to the electrode and bridge device while recording from an ON bipolar cell. The top trace of Fig. 2 illustrates the results of this approach obtained in one cell. A comparatively high dose (1 m*M*) of APB was applied to maximize any conductance changes associated with APB action; the high concentration of APB prolonged recovery, and a return to control recording conditions was not obtained. The application of APB caused a loss of light-evoked activity associated with an increased input resistance as indicated by a larger negative deflection caused by the current pulse. All eight cells tested showed an increased input resistance associated with APB application. Thus the "shunt" mode of action is eliminated: APB seems to be associated with an increase in resistance. An increase in resistance has been proposed as the conductance change associated with the endogenous photoreceptor transmitter (*10*). We wondered therefore whether APB might behave as an agonist on the synaptic receptors of ON bipolar cells. To test this we exposed six ON bipolar cells to 3 m*M* Co^{2+} to block synaptic transmission. All cells responded similarly to that illustrated in Fig. 2 (bottom trace) and showed a depolarization associated with a loss of light-evoked activity. The addition of 500 μM APB caused a rapid hyperpolarization. This experiment suggests that under conditions in which synaptic transmission is blocked, APB directly affects ON bipolar cells and causes a hyperpolarization of the cell.

Electrophysiological evidence indicates that the ON bipolar cell has an unusual synaptic driving mechanism. Receptors release a transmitter in the dark which hyperpolarizes the ON cell. Light stimulation hyperpolarizes photoreceptors, decreases the rate of transmitter release, and depolarizes the ON bipolar cell by an increase in conductance. Thus the action of the endogenous transmitter is to decrease conductance, or close ionic channels. This is in contrast to the action of the photoreceptor transmitter on horizontal cells and OFF bipolar cells, where a more conventional conductance increase has been proposed (*8*, *10*). The increase in input resistance associated with APB could be due to an agonist action of APB on the synaptic receptors of the ON bipolar cells to maintain them in a continuously closed state, unavailable for modulation by fluctuations in endogenous transmitter release. That APB is unassociated with a large change in membrane potential when applied in the presence of normal synaptic function suggests that under the conditions of our experiments (dark-adapted retinas, intermittent light flashes) the endogenous transmitter normally maintains the ON bipolar cell near a maximum level of hyperpolarization. This implies that a large percentage of the synaptic receptors are probably activated.

The compound APB is an analog of glutamic acid and has been used as a glutamate antagonist at invertebrate neuromuscular junctions (*11*) and is mildly excitatory in the cat spinal cord (*12*). Glutamate or aspartate may subserve photoreceptor synaptic transmission, since these excitatory amino acids influence second-order neuronal activity in a manner similar to the endogenous transmitter (*9*, *13*). Therefore, we also examined the effect of the corresponding aspartate analog, 2-amino-3-phosphonopropionic acid. This agent, at concentrations up to 1 m*M*, had no effect on the ON bipolar cell or the ON pathway in general. The results of our study indicate that glutamate is more likely than aspartate to be a transmitter. Our results also strongly suggest that the ON and OFF channels, subserved by different bipolar cells, are segregated according to the specialized synaptic receptors they contain. One type of receptor restricted to the ON bipolar cell appears to be unique among synaptic receptors in the retina, in that activation of this receptor-ionophore complex closes ionic channels. Apparently the selective nature of APB action is due to its special behavior as an agonist that combines with this specialized receptor. Application of APB may be useful in evaluating inner retinal and central nervous connections dependent on ON as opposed to OFF channel input, and may provide additional insight into the molecular configuration of the endogenous photoreceptor transmitter.

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Body Weight and Composition in Laboratory Rats: Effects of Diets with High or Low Protein Concentrations

Abstract. *Adult rats fed high concentrations of dietary protein for 9 weeks gained more weight than rats fed isoenergetic diets containing less protein. There were no significant differences in tail and body lengths among several groups of rats on diets containing different amounts of protein; however, total body fat was significantly greater in the rats fed on diets containing 25 percent protein compared to the rats fed 5 percent protein diets. These findings suggest that the role of dietary protein in obesity and other conditions deserves further scrutiny.*

According to conservative estimates, 30 million adults in the United States are 20 percent or more over their ideal weights (*1*, *2*). Although nutrition is an etiologic factor, at least to the extent that the obese must consume energy in excess of their caloric expenditures (*3*), the role of specific nutrients is unclear. In 1965, the typical American diet was found to contain 16.1 percent of kilocalories as protein from mixed animal and vegetable sources (*4*). This amount exceeds both the daily protein requirement (7.0 percent) estimated by the Food and Agricultural Organization (*5*, p. 74) and the National Academy of Sciences-National Research Council (*6*) recommended dietary allowance for men (7.6 percent). Although both excess and inadequate intakes of energy are potentially harmful, an abundance of protein is generally not considered to be an undesirable feature of the American diet, and harmful effects have not been demonstrated in humans consuming well above the probable requirement.

The aging laboratory rat is a useful model of human obesity and has been used extensively for studies of metabolic changes associated with obesity at the cellular level (*7*). In a study by Edozien and Switzer (*8*) that was confirmed by Donald (*9*) the body weights of rats increased with increasing dietary protein when the rats were fed specially prepared diets from the time they were weaned. However, under the conditions of these experiments, growth was affected (*8*, *9*). In the experiments described here we tested the possibility that body weight and adiposity are influenced by dietary protein in adult animals. We fed a specially prepared diet with varying concentrations of protein to rats that had already achieved full growth.

As shown in Fig. 1, mature rats fed a diet with a high protein content gained weight more rapidly than those fed a low protein diet; linear growth was not affected. Although we did not measure adiposity directly, we speculated that rats fed higher protein diets are more obese than rats fed lower protein diets, because of their resemblance to the spontaneously obese old rat whose adipocytes are large compared to those from young lean rats (*7*). It is noteworthy that commercial laboratory rat food contains 23 percent of protein from mixed animal and vegetable sources, which is equivalent to 18.4 percent as lactalbumin (*10*).

We also fed two groups of rats diets containing either 5 or 25 percent protein for 8 weeks and measured food consumption, body weight, total body fat, and fat-free body mass (FFBM) (Fig. 2). The group that received the 25 percent protein diet had a mean 23.9 percent of its total body mass as fat, whereas the group that received the 5 percent protein diet had a mean of 15.8 percent of total body mass as fat. Final body mass (after removal of the hair and gastrointestinal tract contents) of the rats fed the 5 percent protein diet was 397.3 ± 8.3 g (mean ± standard error) and of the rats fed the 25 percent protein diet was 486.6 ± 9.0 g (t = 7.3, α = .0001). Fat-free body mass was 334.1 ± 12.2 g and 371.0 ± 28.2 g in the 5 and 25 percent protein groups, respectively; this difference was not significant (t = 1.22, α = .1). Total body fat was significantly different between the two groups (t = 2.01, α = .05). The mean value was 63.2 ± 11.7 g in the group fed 5 percent protein and 115.1 ± 23.3 g in the group fed 25 percent protein. These data do not exclude the possibility that the rats fed the 25 percent protein diet have a greater

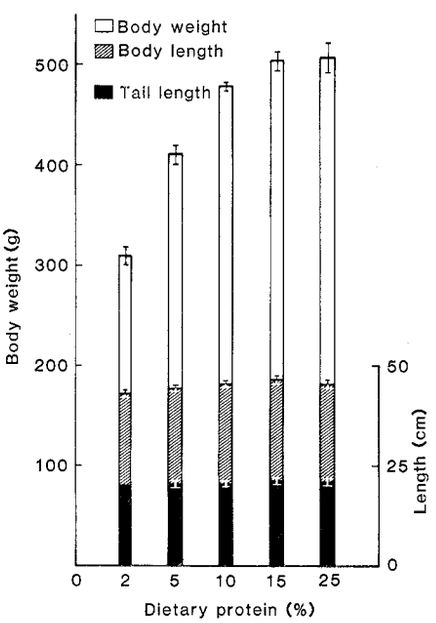

Fig. 1. Body weight, body length, and tail length in mature rats fed diets containing high or low concentrations of protein. Male Sprague-Dawley rats (Hilltop) aged 10 weeks and weighing 300 g at arrival were given free access for 9.5 weeks to isoenergetic (4.1 kcal/g) pelleted diets (Teklad) containing 2, 5, 10, 15, or 25 percent protein (as lactalbumin) with correspondingly decreasing amounts of carbohydrates (as sucrose, cornstarch, and cellulose) and 10 percent fat (as cottonseed oil). There were three rats, housed together, in each dietary group. Body length did not change significantly during the experiment. All animals were fasted for 16 hours and then killed in the morning with an overdose of sodium pentobarbital. We then measured body weight, body length (nose to tail), and tail length. To analyze the differences between the mean variables from the five dietary groups we used the one-way analysis of variance (ANOVA) (*16*). Differences between mean initial and final body weights of each group were determined by the t-test (*5*, p. 71). There were no significant differences in mean body weights among the five groups at the beginning of the experiment (F = 0.21). However, by the end of the feeding period, body weights of the five groups varied significantly (F = 855.5, α = .01). Throughout the study, animals fed the 5, 10, 15, and 25 percent protein diets appeared healthy and well-nourished. The rats in each of these four groups had gained significant amounts of weight since the initiation of the feeding period (t = 4.9 to 22.6, α = .01), whereas the rats in the group fed the 2 percent protein diet had lost an average of 13.8 g (t = 0.8, not significant). However, there were no significant effects of dietary protein on tail (F = 1.89) or body (F = 1.44) lengths.

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