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Light-Flash Physiology with Synthetic Photosensitive Compounds

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I. INTRODUCTION

This review treats a technique that has proved useful recently in some physiological studies. A process is measured while a light flash is used to manipulate a physiologically important or pharmacologically active photosensitive molecule. Typically such experiments complement more common measurements of *1*) equilibrium responses in the same system or *2*) relaxations after voltage jumps, rapid mixing, and so on. The chief advantage of the light-flash technique is the speed of the photochemistry and the fact that it can be applied to organized systems, such as muscle fibers and membranes under electrophysiological investigation, that cannot be flowed. Experiments may be classified by the accessible time scales (Table 1), by successful recording techniques (Table 2), and by available molecules (Table 3).

TABLE 1. *Recording techniques that have been used with light-flash physiology*

Recording Techniques	References
Tension	63–66, 127, 153
Turbidity	121
Metallochromic dye	147
Radioactive tracer fluxes	56, 92
X-ray diffraction	67, 76
Intracellular microelectrode	
(*Electrophorus* electroplaques)	5
Transcellular voltage clamp	
(*Electrophorus* electroplaques)	100, 166
Two-electrode voltage clamp	187
Sucrose-gap voltage clamp (frog heart)	127, 129, 137
Patch clamp	
Whole-cell mode	
Rat myoball	26, 27
Rat cardiac myocyte	70
Rat sympathetic ganglion cell	69, 70a
Aplysia bag cell	134
Single channels	26, 27, 30

TABLE 2. *Time constants of physiological phenomena explored with light-flash physiology*

Time Constants	References
10 μs–1 ms	
Start of channel opening with Bis-Q*	100
Phase 1 with Bis-Q (molecular rearrangement)	27, 131, 166
1–10 ms	
Concentration-jump relaxations (agonists)	26, 27, 110, 114
Open-channel blockade	112
Recovery of Ca current after photoremoval of nifedipine	70, 137
Ca activation of K channels (nitr-5)	69, 70a
Tension transients with caged ATP in striated muscle	64, 65
10 ms–1 s	
Phase 3 with Bis-Q	131
Relaxation of muscarinic conductance	129
Concentration-jump relaxations (antagonists)	101
Na movement in turnover of Na pump	56
Ca binding to sarcoplasmic reticulum with caged ATP	147
Tension transients with caged ATP in striated muscle	64, 65
1–100 s	
Phase 4 and densitization with Bis-Q	131
cAMP-induced increase in cardiac Ca currents	130, 138
Ca uptake by sarcoplasmic reticulum with caged ATP	148
Tension transients in smooth muscle with caged IP_3	66

* This is a measurement of latency to the first detectable signal, rather than time constant.

TABLE 3. *Design versus discovery*

Design Molecules	References
Molecules designed for other purposes and exploited for photosensitivity	
o-Nitrobenzyl cAMP and cGMP	47, 48, 130
Nifedipine, nisoldipine	70, 127, 137, 159
Molecules designed and used for photochemistry	
Bis-Q	5, 26, 27, 110, 129
QBr	5, 6, 30, 114
n,p-Phenylazophenyl carbamylcholine	9, 112
2BQ	101, 194
4,5-dimethoxy-2-nitrobenzyl cAMP, 4,5-dimethoxy-2-nitrobenzyl cGMP	138
Caged ATP	63, 93, 121
Nitr-2	185, 187, 200
Nitr-5	69, 70a
2-Hydroxyphenyl-1-(2-nitro)phenylethylphosphate	96
Caged IP_3	66
Molecules designed for photochemistry and used for other purposes	
o-Nitrobenzyl acetate	177
Bis-Q	193

Bis-Q, 3,3'bis-[α-(trimethylammonium)methyl]azobenzene; QBr, 3-(α-bromomethyl)-3'-[α-(trimethylammonium)methyl]azobenzene; 2BQ, 2,2'-bis-[α-(trimethylammonium)methyl]azobenzene; IP_3, inositol trisphosphate.

One could imagine a general way of performing light-flash studies. A physiologically important but photostable molecule would be placed inside a universal photosensitive cage of molecular dimensions (like the one pictured in Fig. 1 and named *Polytechnus*). The caged molecule could then be introduced near or within the cell of interest, and a flash would liberate the molecule. Although *Polytechnus* would not enable direct photochemical manipulations of a ligand-receptor complex, such a molecule would allow flash-induced concentration-jump experiments with any molecule of interest.

Unfortunately, *Polytechnus* does not yet exist. Therefore, the chief disadvantage of the light-flash technique is that special photosensitive molecules need to be designed or found for each application. This review is thus aimed at researchers considering whether the effort of a light-flash measurement is justified in their own laboratories. An earlier review treated additional topics, such as quantitative aspects of photochemistry, possible unexplored photochemical tactics, and known photosensitive molecules that have not been exploited in light-flash experiments (115).

II. PHOTOCHEMICAL STRATEGIES

Only two types of photochemical reaction account for nearly all the experiments in the light-flash physiology field (Fig. 2). These two reactions are, however, vastly different in many respects.

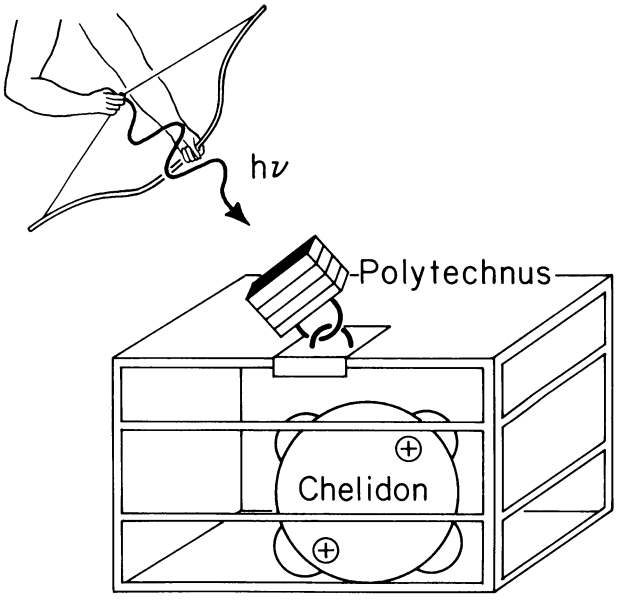


FIG. 1. A hypothetical universal photosensitive cage of molecular dimensions. This molecule would allow the experimenter to render any molecule light activated by trapping it within the cage until released by a photon. One would not have to synthesize a specific photosensitive ligand for each application. We named the hypothetical compound *Polytechnus*, after the mythical Greek who entrapped the lovely Chelidon until she was freed by an arrow from Artemis's quiver. In our rendition of the myth, Artemis shoots photons and Chelidon is a Ca ion, although it might be any other physiologically important molecule. Indeed, the Ca^{2+} complex of *Polytechnus* has recently been rendered obsolete by nitr-5, synthesized by R. Y. Tsien and colleagues.

A. Photoisomerization of Azobenzenes and Related Molecules

Many azobenzene compounds have photophysical properties well suited for experiments on membranes and proteins. The useful molecules exist as distinct *cis* and *trans* isomers, interconversions between the two forms being achieved on exposure to light at appropriate wavelengths. Extensive work has been performed with such photoisomerizable compounds for research on acetylcholine receptors (115).

The closest approach yet to the mythical *Polytechnus* has been achieved with azobenzene-based compounds. A series of amphipathic molecules was synthesized with long-chain, lipid-soluble tails and azobenzene head groups. Because the charge density of the azobenzene group changes with the configuration, the *cis* and *trans* isomers have different critical micelle concentrations (170), and it should be possible in principle to destroy the micelle with a flash. In another promising study, lipids were synthesized containing an azobenzene group within the hydrocarbon chain. Liposomes made from the *cis* form of this molecule are significantly more permeable to ions (as measured by osmotic shrinkage) than those with the *trans* configuration (91, 91a). Another molecule

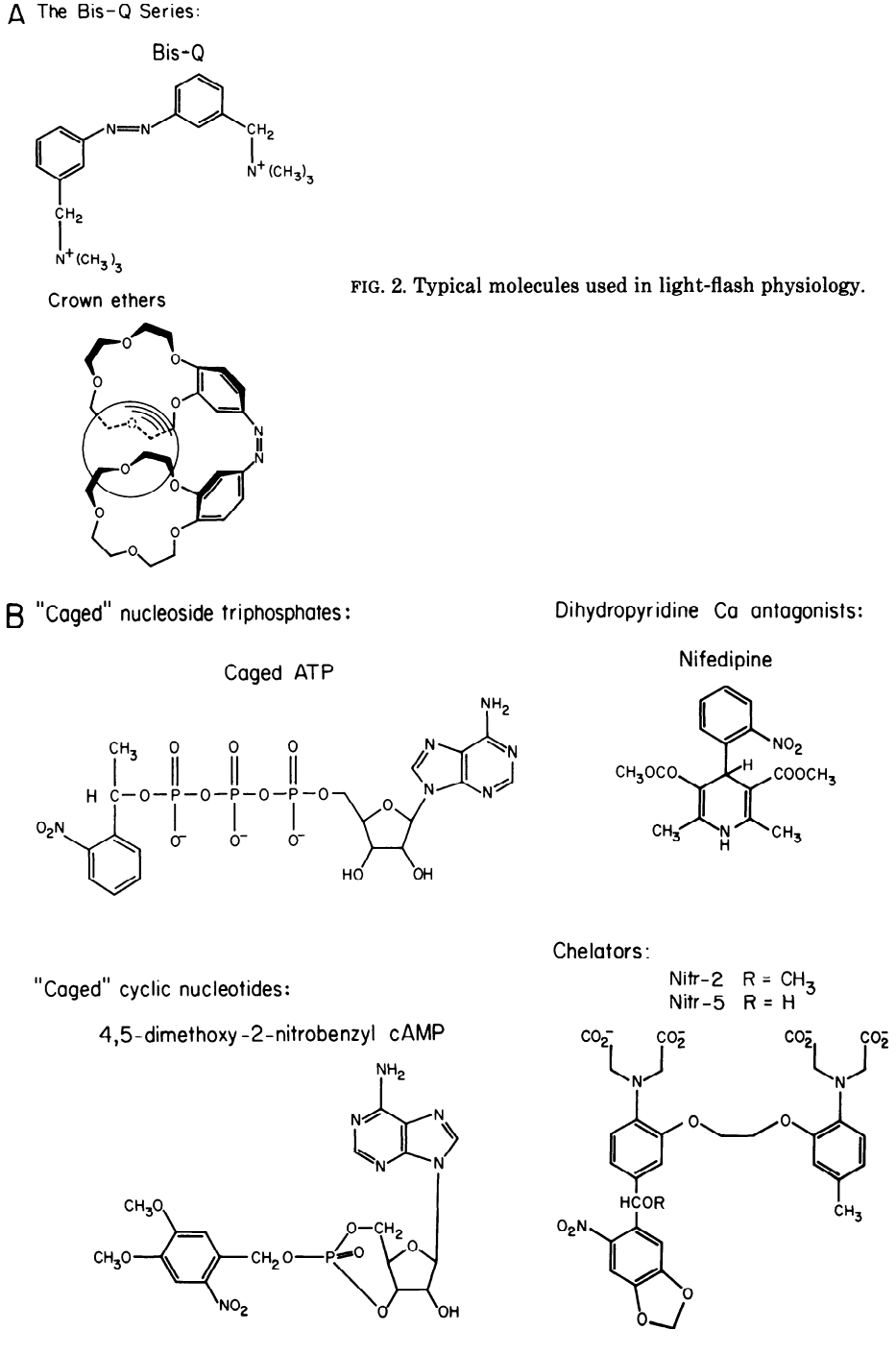


FIG. 2. Typical molecules used in light-flash physiology.

was synthesized containing two cylindrical macrocycles linked by two azobenzene "pillars." With this molecule, it was possible to demonstrate photosensitive inclusion compounds (168). Can *Polytechnus* be far away?

The search for photosensitive chelators has encouraged some creative work. These molecules chelate with different affinities based on their configuration; they should be distinguished from the more usual optical indicators that change their absorption or fluorescence depending on the state of chelation. The most straightforward approach is to exploit the change in shape between *cis* and *trans* azobenzene to modify the placement of the coordinating atoms. This tactic was employed in an azobenzene-based photosensitive zinc chelator (12) and in crown ethers (reviewed in 169; see also 168, 171). A potentially more subtle approach is to exploit changes in the electronic structure of the aromatic rings, themselves resulting from the photoisomerization. A pitfall of this approach is that the azo bond loses its double-bond characteristic in one of these states, and the molecule cannot be stably driven between states by light alone. Shinkai, Manabe, and their colleagues (167–171) have partially overcome these difficulties with a series of compounds based on azopyridine photoisomerization. One such "photoresponsive cryptand" reveals a sixfold difference in its affinity for Cu^{2+} as well as clear differences in the pK_a of the two configurations (167, 170). However, high-affinity photosensitive Ca^{2+} chelators are presently most interesting to physiologists, and there have been no successful azobenzene-based molecules of this class (reviewed by 115). The best available photosensitive Ca^{2+} chelators are now based on *o*-nitrobenzyl derivatives.

A few other photoisomerizable compounds have interesting effects on biological membranes (reviewed by 115). Investigators should be aware, however, that most known azobenzene dyes are not photoisomerizable. Indeed, the dye industry's major requirement is for dyes that are not photosensitive. This goal is generally approached by using electron-withdrawing *para*-substituents that give the azo group a single-bond character, which as noted above generally destroys stable photoisomers.

B. Photolyzable Esters

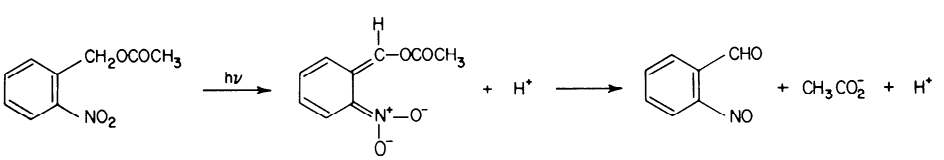
There has been extensive recent interest in the photochemically removable *o*-nitrobenzyl group and its derivatives (see Table 1). This technique has been used to form carboxylates, phosphates, phosphodiesters, amines, alcohols, and ketones (115).

Research with *o*-nitrobenzyl derivatives has focused on the mechanism of photolysis, with a view toward improving the speed, efficiency, and action spectrum of the reaction. Most photochemists find the time scale of the reactions ($\sim 10^{-3}$ s and longer) of little interest, and therefore the development work has taken place primarily within the biophysical community. The pioneering kinetic investigation of caged ATP (93) by McCray et al. (121) es-

tablished the existence of an *aci*-nitro intermediate during photolysis and showed that this intermediate decays in an acid-catalyzed step; at normal pH the decay is rapid enough ($\sim$100 s^{-1}) to enable a fascinating series of kinetic studies on muscle contraction (63–65), although a 10-fold faster photolysis would eliminate some complications in the interpretation of the data. McCray and Trentham (personal communication) are continuing their investigations on the mechanism of caged ATP photolysis and have additional evidence that the rate of breakdown of the *aci*-nitro intermediate does indeed reflect the rate of photorelease (64). Similar mechanisms appear to be involved in the breakdown of an *N*-*o*-nitrobenzyl derivative of carbamylcholine (191a).

Nerbonne (133) conducted flash photolysis studies on a series of *o*-nitrobenzyl esters and on derivatives with various substituents on the aromatic ring or the benzylic carbon. In most of the molecules studied, an *aci*-nitro intermediate was detected with absorption at 410 nm, where the starting material absorbs little. However, with the 4,5-dimethoxy-2-nitrobenzyl acetate, 6-nitropiperonyl acetate, and 2,6-dinitrobenzyl acetate, there was no detectable intermediate within the time resolution of the apparatus (5 ms) at pH 7. This could suggest that the *aci*-nitro intermediate has the same absorption at 410 nm as the breakdown product. At pH 12, however an intermediate with a distinct absorption spectrum could be resolved (decay rate 2–12 s^{-1}). It seems more likely therefore that the breakdown of the intermediate is too fast to measure at pH 8 but is slowed appreciably at pH 12, in accord with the observation (121) that the breakdown of the intermediate is acid catalyzed.

Nitrobenzyl esters liberate a proton on hydrolysis. Therefore Nerbonne (133) conducted a further series of experiments based on measurements of the "pH jump" during photolysis. The efficiency of photorelease is rather high (0.25–0.5) and varies little with the structure of the protecting or leaving group, with the pH of the solvent, or the dielectric constant or ionic strength of the medium. In each case, the release of protons appeared to be complete within 5 ms. The following reaction mechanism, illustrated for the parent molecule, is the most likely, although other mechanisms of proton release are possible (133).



It would clearly be desirable to have more direct kinetic data on the photolysis of *o*-nitrobenzyl derivatives. Nevertheless, at present enough details are known to suggest that further light-activated reagents be based on the procedures suggested by McCray et al. (121) and by Nerbonne (133); i.e., electron-donating substituents on the *o*-nitrobenzyl nucleus can provide a gen-

erally useful protecting group for a variety of functional molecules. A further advantage of these derivatives is that the absorption maximum occurs at 350 nm, and appreciable photolysis can be achieved at wavelengths up to 400 nm, eliminating the need for ultraviolet (UV) grade optics (138).

III. CAGED NUCLEOSIDE TRIPHOSPHATES

Many ATPases participate in relatively complex enzymatic reactions, and their full description calls for a knowledge of the order and timing of the various steps in the complete reaction. Dynamic measurements are crucial for such explanations. Where the energy of the phosphate bond will be converted into the energy of mechanical movement (for example, muscle contraction) or an electrochemical gradient (for example, ion pumping), measurements must be conducted on the system in a nearly natural, ordered state. The photolysis of caged ATP allows for such measurements. Potential users of the technique should be aware that Trentham and his colleagues have synthesized several other members of the "caged family," e.g., ADP, 1,N^6-etheno ATP (40), GTP, and caged inositol 1,4,5-trisphosphate (IP_3) (66); the latter synthesis involves a general approach to caging any phosphate ester containing a weakly acidic group. These molecules will undoubtedly find use in characterizing many systems.

A. Muscle Research

In a typical experiment with caged ATP, a muscle fiber is demembranated by one of several mechanical or chemical means. The fiber is then exposed to caged ATP which equilibrates within the myofibrils. A laser or flashlamp pulse converts some (up to 50%) of the caged ATP to ATP and 2-nitrosoacetophenone. The ATP binds to the cross bridges resulting in transients of tension and stiffness as the cross bridges detach, attach, and generate force. The experiments of Goldman et al. (64, 65) correlated these transients with measurements on the steady-state ATPase activity of isolated contractile filaments.

If this experiment is conducted in the absence of Ca^{2+}, tension and stiffness decay within a few tens of milliseconds (64). In the presence of Ca^{2+}, active contraction is initiated (65). These qualitative phenomena are expected from previous experiments on the control of muscle contraction. From results with varying ATP concentrations ([ATP]), it was concluded that ATP binding and detachment of the cross bridge proceeds with a Ca-independent rate constant of at least $5 \times 10^5\ M^{-1} \cdot s^{-1}$, in agreement with solution measurements on actomyosin. Cross-bridge detachment is slowed by 0.02–1 mM ADP, suggesting an additional mechanism for regulating the contraction (39).

An interesting feature of the tension records in the absence of Ca is a hump (or shoulder) of tension superimposed on a steady decline with time

after the pulse of ATP. This effect is ascribed to a brief reattachment of the cross bridges, which produces a tension increase. Apparently rigor links (actomyosin with no bound nucleotide) remove the tropomyosin inhibition of cross-bridge attachment (18, 19). Earlier evidence for this view is the biphasic curve of tension versus Mg^{2+}-ATP concentration (reviewed by 64).

In the presence of Ca^{2+}, the brief decrease of tension is followed by an increase to a steady level of tension (65). The tension records in the presence and absence of Ca^{2+} begin to separate ~15 ms after the flash, providing a time estimate for the onset of Ca-dependent force generation and showing that Ca does not affect the rate of ATP binding and detachment of the cross bridges. The subsequent rise in tension does not depend on the [ATP], suggesting an intramolecular step with a rate constant of ~80 s^{-1}.

The experiments described above have been repeated in the presence of 10–25 mM inorganic phosphate (79). In the absence of Ca, the relaxation is accelerated; in the presence of Ca, the development of tension proceeds with an unchanged rate but the steady-state level is lower. These observations suggest that a reversible ATPase step is involved in the dominant force-generating cross-bridge state. Thus the use of caged ATP allows the description of events in muscle contraction with close reference to the working of the organized system.

Time-resolved X-ray diffraction measurements have been reported on insect flight muscle (67). The recordings used the synchrotron radiation source at the Deutsches Electron Synchroton in Hamburg. Glycerol-extracted muscles were exposed to caged ATP, and reflections were measured following a flash from a xenon flash lamp. Measurements could be made with a time resolution of 1 ms, and the transient structural changes decayed with a time constant of 8.2 ms, or at least 10-fold faster than the tension transients. This promising new technique will surely yield new and interesting information about the kinetics of muscle contraction.

B. Active Transport Research

Caged ATP was first developed for work on the Na^+-K^+-ATPase of erythrocyte membranes, because the light-flash technique enables one to protect the ATP from endogenous ATPases within the compartment of choice until the desired moment (93). This capability was exploited by Kaplan and colleagues (92–94) to describe details of the ATP-ADP exchange reaction as affected by the intracellular and extracellular concentration of monovalent cations. The major conclusion was that Na displayed similar apparent intracellular and extracellular affinities for regulation of two processes: on the one hand, the Na-Na exchange; and on the other, the ATP-ADP exchange. Furthermore, ATP-ADP exchange could occur in the absence of external Na (92, 94).

Forbush (56) has used caged ATP in transport measurements on the Na^+-K^+-ATPase from kidney. Sodium movement was measured in a single turnover

of the pump by using less-than-stoichiometric release of caged ATP with a rapid filtration device (modified from a phonograph turntable!). $^{22}Na^+$ release from the vesicles rises to a peak within about 100 ms, then decays with a rate constant of 6 s^{-1} at 15°C. This time course is best described in terms of a model in which such release follows closely on the ATP binding reaction. These careful measurements also revealed that caged ATP binds competitively to the enzyme with an affinity roughly 100 times less than that of ATP itself. Caged ATP thus joins the ranks of those "imperfect" light-activated ligands that interact with their targets even before irradiation (115).

Calcium transport also involves an ATPase. Caged ATP has recently proved helpful in studies on the Ca pump in sarcoplasmic reticulum from muscle (13, 147, 148). These investigations were made possible by the convergence of three techniques: time-resolved X-ray diffraction (76), metallochromic dye monitoring of Ca^{2+} concentration (147), and of course rapid photolytic release of caged ATP. Furthermore, structural information can best be obtained from oriented multilayers of stacked, flattened sarcoplasmic reticulum vesicles. These multilayers do appear to behave kinetically in much the same way as more usual vesicle preparations from sarcoplasmic reticulum (148). Over a wide temperature range, Ca was taken up by the vesicles and multilamellar stacks in two phases (rate constants of 60 s^{-1} and ~1 s^{-1} at 25°C). The first phase occurs on the same time scale as the formation of phosphorylated enzyme (as measured by quench-flow experiments); this and other evidence suggests that this phase is a direct interaction between the Ca ions and the pump protein, perhaps via an energy-dependent increase in Ca affinity. The second phase is the bona fide translocation of Ca into the vesicles; it displayed a stoichiometry of 1 mole of ATP hydrolyzed per 2 moles of transported Ca^{2+}. The present challenge is to correlate these Ca movements with the time-resolved changes in diffraction that indicate an ATP-dependent shift of electron density from the vesicular surface of the membrane to the lipid core. The ultimate goal is a motion picture of the transport protein as it undergoes the conformational changes involved in Ca transport.

IV. CAGED CYCLIC NUCLEOTIDES

A. Initial Experiments

Cyclic nucleotides are important intracellular messengers in many systems. Cyclic AMP (cAMP) mediates such diverse activities as the effect of β-adrenergic agents on the heart (152, 186, 190), 5-hydroxytryptamine (5-HT) on *Aplysia* neurons (97, 172), and an electrical afterdischarge in the neurosecretory bag cells of *Aplysia* (89, 90). The intracellular concentrations of cyclic GMP (cGMP) are altered as a result of activation of various receptors, particularly that for atrial natriuretic peptides (149, 191). Cyclic GMP is also involved in the response of vertebrate rod cell photoreceptors to light (31, 52,

74b, 119). To study the effects of cyclic nucleotides, it is necessary to impose artificial changes in their intracellular concentrations. There are many analogues of cAMP that allow such manipulations to be made, and cAMP can also be injected into cells by ionophoresis. Such modulations have proved to be more difficult with cGMP (47), thus its effects are less well understood. Even with cAMP, however, the intracellular concentration can be elevated only slowly, limiting kinetic studies of its effects, and the exact intracellular concentrations achieved are unknown. Light-sensitive "caged" cyclic nucleotides improve the time resolution of studies of cyclic nucleotide effects and should permit known and controllable changes in their intracellular concentrations.

The photolabile cyclic nucleotides, *o*-nitrobenzyl cAMP and cGMP, were developed about the same time as caged ATP as a means of transporting cAMP and cGMP into cells (47, 48). The substituted benzyl triesters are very lipophilic and easily cross membranes. Once in the cytosol, the free cyclic nucleotides might then be liberated either by spontaneous hydrolysis, the action of intracellular enzymes, or photolysis. For an ideal photoactivatable cyclic nucleotide, release of the active group by the former two mechanisms should be negligible so that, in the absence of light, basal levels of cyclic nucleotide are unaltered. On photolysis, release of the free cyclic nucleotide should then proceed rapidly, on the millisecond time scale. The development of the *o*-nitrobenzyl cyclic nucleotides, as well as the studies that determined their suitability as photolabile precursors of cAMP and cGMP (47, 48), have been described in an earlier review (115) and will not be discussed here.

The low rate of hydrolysis of *o*-nitrobenzyl cAMP, along with the inactivity of the hydrolysis byproduct at the low concentrations appropriate for cAMP research, render this molecule suitable as a photolabile precursor for the intracellular accumulation of cAMP in guinea pig papillary muscle (98) and in bullfrog atrial muscle (130). During 5- to 10-min exposure to *o*-nitrobenzyl cAMP (<40 μm), the waveform of the action potential recorded from bullfrog atrial trabeculae was unaltered, although there was a small (<5%) increase in the peak amplitude of the underlying slow inward Ca current (I_{si}), which presumably arose, at least in part, from intracellular hydrolysis of the ester. Release of cAMP or cGMP from the *o*-nitrobenzyl esters is greatly accelerated by exposure to light of wavelengths 300–350 nm (48). Photolysis in solution yields *o*-nitrosobenzaldehyde along with a proton and the cyclic nucleotide itself. A light flash presented to isolated strips of bullfrog atrial muscle, in the presence of *o*-nitrobenzyl cAMP, enhanced the amplitude of I_{si} by up to threefold, resulting in a broadened action potential (130). The earliest increases in I_{si} were observed within 150 ms after the flash. The current continued to increase over the next 30–40 s, reaching a maximum and then eventually recovering toward the initial level after a few minutes, permitting repeated flashes. Responses to flashes lasted longer at higher drug concentrations, in the presence of phosphodiesterase inhibitors and at lower temperatures. The wavelengths required for the increase in I_{si} corresponded to

those necessary for photolysis of *o*-nitrobenzyl cAMP. Preirradiated solutions of the photolabile analogue were without effect, and flashes were only effective in the presence of *o*-nitrobenzyl cAMP. Only small nonspecific flash effects were observed in the absence of drug or in the presence of 500 μM *o*-nitrobenzyl cGMP or 100 μM *o*-nitrobenzylacetate, confirming that the physiological effects of flashes arose from an increase in the intracellular cAMP concentration and not from changes in intracellular pH associated with photolysis or from an action of the nitrosobenzaldehyde byproduct.

B. Newer Derivatives

The *o*-nitrobenzyl esters of cAMP and cGMP do have some drawbacks as photochemically caged molecules.

1) Although hydrolysis of the esters in solution is slow, some basal release of the free cyclic nucleotides does occur (48, 98), resulting in small but detectable changes in cellular physiology, even before photolysis (98, 130).

2) The photolytic release is relatively slow with a time constant of hundreds of milliseconds (133).

3) Although the efficiency of photorelease of the cyclic nucleotides is relatively high, cGMP is released with only half the efficiency of cAMP. This might be due to competing secondary side reactions during photolysis of the cGMP derivative.

In her systematic study of the structural factors that determine the rate and efficiency of photorelease from photochemically caged molecules, Nerbonne (133) found that the 4,5-dimethoxy-2-nitrobenzyl esters of cAMP and cGMP had more favorable properties. Irradiation yields 4,5-dimethoxy-2-nitrosobenzaldehyde, a proton, and the cyclic nucleotide itself. Photorelease from these analogues is fast, efficient, and similar for the cAMP and cGMP derivatives (133, 138). In the dark, these molecules are physiologically inert, up to a concentration of 200 μM in bullfrog atrial trabeculae. They hydrolyze very slowly in solution, with a half-life of 36 and 100 h for the axial and equatorial isomers, respectively. The photochemical reactions that liberate the free cyclic nucleotides seem to be complete within at most 5 ms (although an alternative explanation of the data is given in sect. II*B*), which is $\sim$200 times faster than photorelease from the unsubstituted *o*-nitrobenzyl derivatives. The absorption maxima of the dimethoxy derivatives are at 350 nm, which is significantly red-shifted from that of the simple *o*-nitrobenzyl esters. The action spectrum is shifted as well, so that longer wavelengths, which are less likely to damage cells, can be used to trigger photolysis. In addition, under the same experimental conditions, the efficiency of photorelease of cyclic nucleotides is twice as great with the newer derivatives, and it is uncomplicated by competing secondary side reactions. Thus lower concentrations of the dimethoxy derivatives can be employed to produce equivalent cyclic nucleotide concentration "jumps." The efficiency of photorelease is also inde-

pendent of ester concentration, the ionic strength, pH, or dielectric constant of the medium.

C. Heart Muscle

In the presence of 2–100 μM 4,5-dimethoxy-2-nitrobenzyl cAMP, flashes produce large increases in the amplitude of I_{si} recorded from bullfrog atrial trabeculae with no measurable changes in the kinetics or voltage dependence of the current (138, 153). They also cause broadening of the action potential (138, 153) and an increase in the action potential frequency of spontaneously beating frog sinus venosus fibers (59). Studies of the effects of flashes in this preparation confirmed the results obtained previously with the unsubstituted *o*-nitrobenzyl derivative. Even with many experimental improvements, including the efficiency and rate of release of cAMP from the new molecules, increases in I_{si} were not observed at times earlier than 150 ms after the flash. Over the next few seconds after the flash, the peak of I_{si} increased linearly with time; when the data were extrapolated to zero increase, the results indicated that there was no detectable delay after the flash, with a time resolution of 100–200 ms. These data show *1*) that the light-flash method of cAMP application is not yet capable of resolving steps with a time constant of 100 ms or faster in this preparation, and *2*) that on a longer time scale, there seems to be a single rate-limiting step between the elevation of intracellular cAMP concentration and modulation of I_{si}.

Flash-induced concentration jumps of cAMP also resulted in an increase in phasic tension without altering the kinetics or voltage dependence of the tension waveform (153). Tension increased over a parallel time course to the increase in I_{si} amplitude. There was no measurable delay between the onset of changes in I_{si} amplitude and increases in tension (within 100–200 ms resolution), thus implying a direct correlation between tension and Ca influx through Ca channels in the sarcolemma. It was concluded that all of the effects of cAMP on phasic tension may be due to the enhanced influx of Ca and that the experiments did not reveal any direct effects of cAMP on processes involved in the development of, or recovery from, phasic tension. The results therefore support the general conclusion that the enhancement of I_{si} results from the direct phosphorylation of the Ca channel protein (36).

Tonic tension, which develops during depolarizing voltage steps after blockade of I_{si} and is thought to result from the influx of Ca via electrogenic Na-Ca exchange, was also influenced by changes in intracellular cAMP concentration (153). Cyclic AMP concentration jumps reduced tonic tension while increasing the amplitude of the outward delayed rectifier current (I_K). The time courses of development of these two effects were, however, poorly correlated. They were also poorly correlated with the time course of the effects on the action potential, I_{si}, and phasic tension, suggesting that a single mechanism is unlikely to account for all the effects of cAMP on bullfrog atrial

cells. Photoinduced cAMP jumps thus revealed that, in frog atrial muscle, cAMP may produce its effects by modulating multiple processes, which vary in their kinetics and sensitivities to cAMP concentrations. It seems likely that detailed mechanistic studies of the various processes underlying the effects of cAMP will be possible with this photochemical approach.

In contrast to the observations in the presence of photolabile analogues of cAMP, flashes were without effect on cardiac muscle exposed to 500 μM *o*-nitrobenzyl cGMP (130) or 4,5-dimethoxy-2-nitrobenzyl cGMP (138, 153). Neither did cGMP analogues have any effect on flash-induced responses in the presence of the cAMP derivatives (130, 153). These experiments should now be repeated under the condition whereby cGMP decreases Ca current by activating a cAMP phosphodiesterase (74a).

D. Other Tissues

Photolabile cAMP derivatives have also been used to study the possible involvement of cAMP in drug-induced inhibition of platelet aggregation caused by thrombin (10). 4,5-Dimethoxy *o*-nitrobenzyl cAMP ($\leq$200 μM) had little effect on thrombin-induced aggregation of platelets from rat blood. Preirradiated caged cAMP solutions and flashes (300–350 nm, 1 ms) presented in the absence of photolabile esters were similarly without effect. Flashes presented in the presence of the caged cAMP caused an inhibition of thrombin-induced platelet aggregation and release of 5-HT. The degree of inhibition depended on the amplitude of the flash-induced concentration jump; aggregation was almost completely prevented by flashes causing jumps on the order of 10 μM. The results support the involvement of cAMP in inhibition of platelet aggregation.

Other experiments employing the photolabile caged cyclic nucleotides have, however, been less productive. Ciliary motility in *Paramecium* is thought to be regulated by cAMP (15). Solutions containing caged cAMP (200 μM) are without effect on the behavior of *Paramecium*, but irradiation with light of 300–400 nm, which converted $\sim$5% of the caged molecules in 5 s, caused a marked and rapid (<1 s) change in the direction of swimming, followed by acceleration of forward swimming (R. Gunderson and D. Nelson, personal communication). Light on its own did not influence motility. The experiments were complicated, however, because flashes in the presence of the cGMP derivative and in the presence of *o*-nitrobenzyl acetate also caused behavioral changes, implying that the effect was not specific for cAMP. One must therefore consider the flash-induced pH jump as the cause of the behavioral effects. Experiments with caged cAMP did not give straightforward results in transporting epithelia in which chloride secretion is stimulated by cAMP (37). Low concentrations of caged cAMP caused chloride secretion in rat colon and two cultured cell lines (human colonic HCA-7 and amphibian kidney A6) in the dark, and no additional effects were seen with irradiation. Preirradiated sam-

ples of caged molecules were also effective, and treatment of tissues with a variety of esterase inhibitors did not alter the effect (W. W. Cuthbert and D. J. Brayden, unpublished data).

Caged cGMP was used to study cGMP modulation of the light response in bullfrog rod photoreceptors. Recent reports on the role of cGMP in mediating the dark current recorded from photoreceptors (31, 52, 74b, 119, 124) suggested that flashes presented in the presence of caged cGMP might increase the dark current. Suction pipette recordings were made from isolated outer segments while caged cGMP was present in the bath. Flashes failed to produce the expected result (G. Nicol, A. M. Gurney, D. Bownds, and H. A. Lester, unpublished data). The reasons for this are at present unclear but will hopefully be resolved in future experiments with excised inside-out membrane patches.

Thus, although the caged cyclic nucleotides have been useful tools in some tissues, their widespread usefulness remains to be determined. Several questions still remain about the exact concentrations of the active molecule that are released in the cytosol. If the photolabile esters preferentially partition into membranes, then they could accumulate inside cells and the magnitude of flash-induced jumps could vary locally. As a result it would not be possible to determine precisely the concentration dependence of the response in question. Membrane impermeant analogues might therefore be better suited to quantitative studies of cyclic nucleotide effects.

V. NICOTINIC ACETYLCHOLINE RECEPTORS

A. Electrophorus Electroplaques

Since light-flash physiology was previously reviewed (115), the photoisomerizable competitive antagonist 2,2′-bis[α-(trimethylammonium)methyl]-azobenzene (2BQ) has been employed in a kinetic and equilibrium investigation on the nicotinic receptor of *Electrophorus* electroplaques (101). Competitive antagonism was shown by the nearly parallel right shift in agonist dose-response curves for 2BQ concentrations <4 μM. These data revealed apparent dissociation constants of 0.3 and 1 μM for the *cis* and *trans* isomers, respectively.

Flash-induced *trans*-to-*cis* concentration jumps produced the decrease in agonist-induced conductance expected from these equilibrium data; the time constant was several tens of milliseconds. The concentration dependence of these rates was studied; it was concluded that the association and dissociation rate constants for the *cis*-2BQ-receptor interaction are, respectively, $\sim 10^8$ $M^{-1} \cdot s^{-1}$ and 60 s^{-1} at 20°C.

Molecular rearrangements of the ligand-receptor complex could be produced by *cis*-to-*trans* photoisomerizations. As opposed to earlier data on direct

molecular rearrangements (113, 131, 166), these data were not readily interpretable in terms of molecular rate constants. Instead, because of the very tight ligand-receptor binding, the data were probably dominated mostly by the kinetics of buffered diffusion.

Because this study closed the chapter on light-flash experiments with *Electrophorus* electroplaques in our laboratory, we speculated about the relative rates of agonist and antagonist binding at the nicotinic receptor. There are still some uncertainties, but there now seems to be general agreement that the agonist-receptor interaction proceeds with a forward rate constant of 10^8–10^9 $M^{-1} \cdot s^{-1}$ at 20°C, with bis-quaternary compounds falling in the high end of this range. The study with 2BQ showed that the antagonist-receptor binding proceeds with rates about an order of magnitude smaller. These differences in binding rate are probably small enough to agree with the generally held view that *1*) antagonists and agonists have similar initial steric and coulombic interactions with the receptor's binding site, but *2*) only the latter allow the subsequent conformational changes that open the channel.

B. Cultured Rat Myoballs

In our laboratory at Caltech, we have found that patch-clamp recordings complement the light-flash experiments in a nearly ideal manner (109, 133). Patch-clamp electrodes (see 157) are less susceptible to various electrical and photoelectric artifacts than the high-resistance intracellular electrodes previously used in cellular neurobiological studies with light flashes.

1. The agonist, Bis-Q

In a preliminary electrophysiological survey, it was found that 3,3′bis-[α-(trimethylammonium)methyl]azobenzene (Bis-Q) is an agonist at the nicotinic acetylcholine receptors of cultured chick and rat skeletal muscle and in the clonal mouse cell line, BC3H-1. Primary rat muscle cultures were selected for further investigations. Experiments were enhanced by the availability of the pure *cis* isomer of Bis-Q, which is not an agonist (41, 139). Both macroscopic and single-channel experiments with the reversibly bound photoisomerizable agonist, Bis-Q, and the covalently bound agonist, 3-(α-bromomethyl)-3′-[α-(trimethylammonium)methyl]azobenzene (QBr), conformed to the general pharmacological pattern observed with these molecules at *Electrophorus* electroplaques (although Bis-Q is a poorer agonist in the myoball preparation; see below). For instance, in a typical experiment, a myoball under whole-cell clamp is exposed to a steady submicromolar concentration of *cis*-Bis-Q. Flashes are delivered through the microscope objective used to visualize the electrodes and the cell. A transient bolus of *trans*-Bis-Q, 50–200 μm in diameter and centered on the myoball, thus appears, and these *cis*-to-*trans* photoisomerizations produce increases of the agonist-induced conduc-

tance. In experiments with excised outside-out membrane patches, this conductance increase can be resolved at the level of single channels opened by the flash (26); when such recordings are summed, they approximate the time course of the macroscopic recordings. As the bolus of *trans*-Bis-Q diffuses away and is replaced by nonirradiated *cis*-Bis-Q molecules, the conductance returns to normal. In a variant of this experiment, *trans*-Bis-Q molecules can be photoisomerized to the *cis* state while bound to receptors, and as a result, channels close (27).

For dose-response studies, the flashes are calibrated with regard to their potency for *cis-trans* photoisomerization (26). With the help of filters, the concentration of *trans*-Bis-Q within the bolus can be controlled. The experiments revealed that the half-maximally active concentration of Bis-Q at cultured rat muscle is several tens of micromoles or roughly 100 times greater than at *Electrophorus* electroplaques or at certain fish muscles (194a). At these concentrations, Bis-Q acts as a channel blocker in addition to its agonist action. These complicating effects prevented a complete analysis of dose-response relations.

It was nonetheless possible to explore dose-response relations in the low-concentration range (26). The approximated Hill coefficient for the response to *trans*-Bis-Q was very near 2, in confirmation of the usual idea that the open state of the acetylcholine receptor channel is much more likely to be associated with the presence of two bound agonist molecules than with a single one. The apparent Hill coefficient varied with membrane potential, but this observation probably does not represent a true change in the functional stoichiometry of channel activation with voltage; rather it reflects the fact that the agonist-receptor affinity depends on voltage, so that dose-response curves shift with voltage (111).

Because of technical factors, currents at positive potentials can be more accurately resolved with flash-activated Bis-Q than with other techniques. It therefore became possible to study the voltage dependence of channel activation over a rather wide range of membrane potential (28, 29). The results showed that the rate constant for channel closing is not, as usually supposed, exponentially dependent on voltage but begins to level off at positive potentials. The opening rate is not, as usually supposed, independent of membrane voltage but begins to decrease at positive potentials. The ratio of the two parameters determines the equilibrium level of activation, which depends nearly exponentially on membrane potential.

2. The agonist, QBr

The covalently bound photoisomerizable agonist, QBr, also induced conductances in rat myoballs that resembled those induced in *Electrophorus* electroplaques (30, 113). For these experiments, receptors are first reduced by exposure to dithiothreitol, then exposed to *trans*-QBr. The unreacted QBr is

then washed away, and the "tethered" QBr molecules can be photoisomerized reversibly between the *cis* (active) and *trans* (inactive) conformations, with the expected relaxations of the conductance. Both the light-flash and voltage-jump relaxations yielded a time constant of ~5 ms at −100 mV and 15°C, in good agreement with single-channel measurements with tethered QBr in cell-attached and outside-out membrane patches.

Functional stoichiometry can be tested with QBr by observing the conductance relaxations in response to flashes that produce known perturbations of the *cis*-to-*trans* ratio. These measurements led to the conclusion that channel activation is controlled by the isomeric state of a single tethered QBr molecule at each receptor. This result is different from the usual conclusion with reversibly bound agonists—including Bis-Q, as described above—but it agrees with previous results obtained with QBr at *Electrophorus* electroplaques (113). This type of experiment, in which the drug-receptor complex is directly perturbed, is uniquely possible with the light-flash technique. This is not a concentration-jump experiment and would not be possible with that mythical universal cage, *Polytechnus*.

Single-channel recordings with tethered QBr (as well as with the tethered photostable agonist, bromoacetylcholine) revealed the existence of a population of openings much briefer (~0.5 ms) than the dominant component. Such excess brief openings have also often been seen with reversibly bound agonists and are often interpreted in terms of the well-known requirement for two bound agonist molecules to open the channel; perhaps the brief openings represented a small contribution from monoliganded channels. With reversible agonists, however, the concentration dependence of the brief openings does not completely conform to this hypothesis (33, 103, 173). Because the dominant open state with QBr is already monoliganded, the hypothesis also seems incomplete in this case. Thus an additional mechanism must account for some of the brief openings seen with both reversibly bound and tethered agonists.

C. Future Directions

Photoisomerizable ligands for the nicotinic receptor have continued to yield useful information on the mechanism of receptor activation in the patch-clamp era. After nearly a decade of electrophysiological work in our laboratory, however, we have recently felt that our techniques have reached their limit in coaxing interesting surprises from Bis-Q and its analogues. We are therefore pleased to note that Delcour and Hess (42) have recently reported experiments using quenched-flow measurements with Bis-Q on receptor-mediated fluxes in vesicles from *Electrophorus* electroplaques. Furthermore Walker et al. (191a) have developed an *o*-nitrobenzyl derivative of carbamylcholine that should circumvent many of the pharmacological limitations posed by Bis-Q.

VI. MUSCARINIC ACETYLCHOLINE RECEPTORS

Several studies have shown that there is a delay of at least 100 ms between application of muscarinic agonists to heart cell membranes and the onset of the membrane's response (61, 74, 81, 129). Most studies, however, could not eliminate the possibility that access of the agonist to the receptors was limited by diffusional barriers. This problem could be minimized by using a photolabile agonist of the muscarinic receptor. The ideal molecule would be inactive before exposure to light but would be rapidly converted with a light flash to a derivative with high affinity and efficacy for the muscarinic receptor. This way the agonist would have unrestricted access to the receptor but would not bind until photoconverted to the active form. Two types of photolabile molecule cold be envisaged, either a photoisomerizable molecule or a caged agonist molecule. Unfortunately no light-sensitive agonists of the muscarinic acetylcholine receptor were known until recently (191a). A number of photoisomerizable azobenzene derivatives that act as agonists at the nicotinic acetylcholine receptor turn out, however, to be antagonists at muscarinic receptors in frog atrial tissue (129). Although experience with photosensitive muscarinic agents has advanced little since the description of azobenzene effects in a previous review (115), they are discussed here again in some detail for the sake of providing a more complete overview of light-flash studies. Bis-Q and its 2,2′ analogues (114, 193) as well as *n-p*-phenylazophenyl carbamylcholine (EW-1) and its *N*-phenyl derivatives (9, 112) block the outward potassium current induced in frog atrial trabeculae by muscarinic agonists (129). Each analogue is effective in around the same concentration range, and all are more potent as antagonists when they are in the *trans* configuration than in the *cis*. Bis-Q has been studied more thoroughly than the other molecules, principally because it has been more readily available (129). Binding and electrophysiological studies show very good agreement on several aspects of blockade by Bis-Q. Both approaches find that Bis-Q blocks the muscarinic receptor in both isomeric configurations, although the *trans* isomer, with a dissocation constant of 5 μM, is three- to fivefold more potent than the *cis* form. Blockade of the receptor by both isomers appears to be competitive, whether inhibition measured is of binding of the antagonist [^{3}H]*N*-methyl scopolamine or of the increase in potassium conductance induced by the muscarinic agonist carbachol.

The difference in affinity of the two Bis-Q isomers for the muscarinic receptor has been exploited to study the kinetics of the agonist-receptor interaction, using the sucrose-gap voltage-clamp method to measure agonist-induced potassium currents in frog atrial trabeculae (129). When the preparation was exposed simultaneously to carbachol and Bis-Q, agonist-induced currents could be increased by presenting flashes that promoted *trans*-to-*cis* photoisomerizations. Conversely, *cis*-to-*trans* transitions caused a decrease in the agonist-induced current. Current relaxations in either direction had the same general features. The relaxations followed a sigmoid time course,

with an initial delay lasting tens of milliseconds, and reached equilibrium after several seconds. The high temperature sensitivity of the kinetics rendered it highly unlikely that the long latency between agonist application and the response resulted from buffered diffusion. The results of these experiments placed constraints on the nature of the rate-limiting step, which must occur at some point after agonist-receptor binding, and stressed several earlier implications that there must be at least two intermediate biochemical steps between agonist-receptor binding and the final membrane response. The study did not, however, identify the molecular nature of these steps.

Photoisomerizable muscarinic antagonists could also be useful for studying the activation of muscarinic receptors in other tissues, such as smooth muscle and autonomic ganglia, where the responses also follow a slow time course. Even more information could be gained, however, from a photolabile agonist. Although no known agonists appear to be photoisomerizable, as noted in section V, carbamylcholine has recently been caged with a photolyzable *o*-nitrobenzyl group (191a). This molecule should provide insights into muscarinic receptor activation not easily obtained by more conventional methods and could also prove useful for detailed kinetic studies at the nicotinic receptor.

VII. PHOTOLABILE CALCIUM CHELATORS

The intracellular Ca ion concentration is tightly regulated. Changes in Ca concentration, however, have profound effects on cellular physiology (e.g., 25, 44, 150). The ion plays an important role in many processes including secretion (23, 43), transmitter release (95), and muscle contraction (154), as well as modulating the activities of various enzymes (73, 128, 143, 144, 180) and membrane ionic channels (1, 3, 32, 45, 118, 122, 145). Requirements of enzyme activity for Ca can often be studied in the test tube, and patch-clamp recording techniques (72) allow one to study Ca modulation of ion channels in isolated membrane patches (e.g., 4, 105, 198). To study how various systems are controlled by Ca in intact cells, however, and to understand the kinetics of its action, it would clearly be desirable to have a means of directly manipulating the intracellular Ca concentration on a rapid (μs to ms) time scale.

Several approaches have been taken to find a photolabile molecule that would allow one to produce concentration jumps of Ca inside cells with light flashes (115). Such a molecule would need to satisfy all the criteria previously outlined for a pharmacologically useful intracellular probe (115, 133). In addition, when loaded into cells, such a molecule should leave the basal intracellular Ca concentration unchanged. It should therefore have a dissociation constant not exceeding 0.1 μM, the normal cytosolic concentration of Ca in most cells. To provide efficient release of Ca, the product of photolysis should bind Ca much more weakly, preferably with a dissociation constant at least 100-fold higher (115). As Ca can affect cellular physiology when applied extracellularly as well as intracellularly, a more useful molecule might be a

hydrophilic one. It could not then be loaded into cells by simply letting it diffuse across the membrane, as in the case of the other photolabile intracellular probes described here, but changes in Ca concentration could be more easily controlled on either side of the membrane. Such a molecule might provide more spatially uniform concentration jumps inside cells, as, unlike the strongly lipophilic nucleotides and cyclic nucleotides, it would not tend to accumulate in lipid compartments of the cell. It is also essential that the molecule does not significantly bind magnesium.

The most successful approach so far has been that of Tsien (185), who recently developed a series of photolabile Ca donors derived from the chelating agent [1,2-bis(2)aminophenoxy]ethane *N,N,N',N'*-tetraacetic acid (BAPTA). Nitr-2, the first to be characterized chemically and used in physiological experiments (185, 187, 200), chelates a single Ca ion with a K_D of 160 nM at an ionic strength of 0.1 M. Upon irradiation the 2-nitrobenzhydrol form is converted to a 2-nitrosobenzophenone, which binds Ca 40-fold more weakly with a K_D of 7 μM. Thus free Ca is released from the Ca-nitr-2 complex when it is exposed to near-UV light. The quantum efficiency of photolysis is 0.03 for the free molecule and 0.1 for the molecule complexed with Ca. The release of Ca proceeds with a time constant of ~200 ms, allowing subsecond changes in Ca^{2+} concentration. A single flash from a xenon flashlamp photolyzes about 20% of the benzhydrol under optimal conditions.

Nitr-2 has been used to study Ca^{2+}-activated membrane currents in voltage-clamped single neurons from the abdominal ganglion of the marine mollusc *Aplysia californica* (187, 200). The buffer was introduced into the cells by pressure injection of a 200- to 400-mM solution, 75% complexed with Ca. Flashes, focused from a xenon flash lamp (~200 J in 2 ms) onto injected cells, elicited two voltage-dependent currents identified as a Ca-activated potassium current and a Ca-activated nonspecific cation current. The currents reached a maximum at ~35–40 ms after the flash, surprising in view of the relatively slow rate of photorelease of Ca from the Ca-nitr-2 complex, but perhaps explainable by the peculiarities of optical shielding, buffering, and diffusion in these cells (187).

Slightly different results were obtained when nitr-2 was used to study Ca-activated currents in dissociated rat superior cervical ganglion cells (69, 70a), using patch-clamp recording techniques (72). The whole-cell variant was used to record membrane currents, and the cells were loaded with nitr-2 by including it in the solution inside the patch pipette. Thus the concentration of nitr-2 and Ca could be easily controlled as the cell interior is dialyzed with the solution in the pipette. When ethyleneglycol-bis(β-aminoethylether)-*N,N'*-tetraacetic acid (EGTA) was used as the buffer in place of nitr-2, light flashes had no effect. When nitr-2 (2 mM), 50–75% complexed with Ca, was included in the pipette, flashes produced an outward current at potentials more positive than −20 mV. The amplitude of the current varied with membrane potential in the manner expected of a Ca-activated potassium current. The current increased in an approximately exponential manner with a time constant on

the order of 150–220 ms; it did not appear to vary with the membrane potential. These properties are expected if the rate-limiting step is the release of Ca from the Ca-nitr-2 complex. Thus to probe the kinetics of Ca activation of membrane currents we clearly need compounds that will release Ca on a faster time scale.

A recent development by Tsien's group is a very promising derivative of nitr-2, known as nitr-5. This molecule binds Ca with similar affinity to nitr-2 in the photolyzed and unphotolyzed forms. The photochemical reactions proceed much more rapidly, however, Ca release proceeding with a time constant of ~300 μs (J. Kao and R. Y. Tsien, personal communication). Experiments have begun with nitr-5 in cultured rat superior cervical ganglion cells, with the chelator included in the patch pipette during whole cell recording (69, 70a). When the solution in the pipette contains 10 mM nitr-5, 75% complexed with Ca, flashes produce outward potassium currents that rise with a time constant of several milliseconds at +40 mV. Both the amplitude and time constant of the flash-induced current relaxation depend on the magnitude of the Ca concentration jump and the membrane potential. The relaxations do not appear to be limited by the rate of Ca release from the chelator. Nitr-5 does indeed appear to be a useful photolabile Ca donor, and we may be able to measure directly the rate of opening of Ca-activated channels in response to a Ca "jump."

VIII. CALCIUM ANTAGONISTS

The Ca antagonists nifedipine and nisoldipine were developed during searches for clinically useful agents and incidentally are sensitive to light because they contain *o*-nitrobenzyl groups. Calcium antagonists have been found to be of great value in the treatment of various cardiovascular disorders (75, 142, 174, 199). The mechanisms of action of these drugs have recently been the subject of intense study and of numerous reviews, of which only a few of the most recent are listed (24, 54, 83, 86, 132, 162, 163, 175). They are a structurally diverse group of compounds that inhibit the influx of ions through Ca channels in several tissues. In some cases, they do interact directly with the Ca channel in the plasma membrane, but the detailed mechanisms of blockade are still the matter of much debate (7, 70, 77, 83, 84, 108, 137, 158).

Calcium antagonists are of interest not only as valuable cardiovascular drugs but also as tools for studying the pharmacology and physiology of Ca channels. The dihydropyridine group of Ca antagonists has been particularly useful in attempts to isolate and purify the Ca channel protein (34–36, 123, 151). Although dihydropyridines appear to be selective for the Ca channels in cardiac and smooth muscle cells, they have also been found to bind with high affinity to sites in skeletal muscle (34, 35, 57, 62, 164) and neuronal tissue (34, 68, 117, 123, 197), both of which have been used as sources for Ca channel isolation. Although in some cases Ca antagonist binding sites seem to have

a physiological correlate, this has not been clearly demonstrated in all tissues (123, 164), and the relationship between binding and functional Ca channels is poorly understood. Thus while the isolation and purification of Ca channels proceeds, it is essential that the characterization of Ca antagonist effects in various tissues continues. There are, however, several obstacles to studying Ca antagonist actions. For example, Ca channels in isolated cells appear to be labile, and voltage-clamp studies are frequently hampered by the gradual disappearance of Ca currents during an experiment. This property, known as current "rundown" (20, 99), is highly variable and not well understood. As a result it is often difficult to distinguish between drug-induced suppression of the current and rundown (51), particularly at low drug concentrations. In addition, experiments with organic Ca antagonists are hampered by the very slow reversibility of blockade, possibly resulting from the strongly lipophilic nature of these compounds. This property would normally limit their usefulness in mechanistic studies. Recent results show, however, that the effects of some dihydropyridine antagonists, such as nifedipine and nisoldipine, are rapidly reversed by exposure to light (70, 127, 134, 137, 159), thus making them useful as reversible blockers of Ca channels.

Since its introduction in the early 1970s (53,55), nifedipine has been known as unstable when exposed to light. This property was exploited in an improved fluorometric assay for nifedipine (160). The product of nifedipine photolysis fluoresces more intensely than the parent compound. Ebel et al. (44a) later showed that irradiation of nifedipine for several minutes with a 300-W light source converted it to a nitroso pyridine derivative. The peak of the absorption spectrum of the photoproduct is shifted to shorter wavelengths compared with nifedipine. The wavelengths important for the conversion are 320–450 nm. Similar observations have since been made with the related drug, nisoldipine (159).

Several studies have now shown that these photochemical properties correlate with changes in the potency of nifedipine and nisoldipine as Ca antagonists. The Ca antagonist activity of nifedipine is effectively destroyed following exposure to light of wavelengths 300–450 nm. The photoproducts of nifedipine and nisoldipine have no effect on twitch tension in strips of frog cardiac muscle (127, 137). They also have no effect on the action potential recorded from ventricular tissue(127) or on Ca currents recorded from frog atrial trabeculae (137), calf cardiac Purkinje fibers (159), rat ventricular myocytes (70), and molluscan neurosecretory cells (134). In the case of nisoldipine, however, although the potency is reduced after irradiation, the photoproduct does retain some blocking activity in some tissues (134, 159). Calcium channel blockade by the dihydropyridine derivatives nitrendipine and nicardipine, which lack an *o*-nitrobenzyl group, as well as by the nondihydropyridine antagonists D 600 and diltiazem, is not modulated by light (127, 159). Nimodipine, a *meta*-nitrobenzyl dihydropyridine antagonist, can be photoinactivated, but much longer pulses of light are required for recovery of nimodipine-blocked twitch tension in frog ventricular muscle (127).

A. Heart Cells

The reactions leading to photoconversion of nifedipine are complete within 100 μs (127). Single flashes can thus be employed to create rapid changes in the concentrations of nifedipine and nisoldipine. The rapid time course of photoinactivation was first exploited by Morad et al. (127). We note that in such experiments, the *o*-nitrobenzyl group does not provide a cage for a physiologically active molecule. Rather, the photosensitive compound starts in the active state and is destroyed when the *o*-nitrobenzyl group is removed. Morad et al. (127) confirmed that nifedipine and nisoldipine act by blocking the flow of ions through Ca channels on the surface membrane of ventricular cells by showing that, although short pulses of light markedly enhanced the action potential or twitch tension developed in frog ventricular trabeculae blocked by nifedipine or nisoldipine, they were without effect when the I_{si} was additionally blocked with 2–3 mM Ni^{2+}. In frog atrial strips (139) and isolated rat heart cells (70), in the presence of up to 1 μM nifedipine, a single flash of 1–2 ms duration, delivering 1 J total output energy from a xenon short-arc flash lamp, was sufficient to destroy >90% of the activity of nifedipine within a few milliseconds.

Light-flash studies with nifedipine in isolated heart cells have proved particularly helpful for mechanistic studies of the drug's action (70). The use of whole-cell patch-clamp techniques (72) with this preparation allowed I_{si} to be studied in relative isolation from other membrane currents. It also eliminated any diffusional problems associated with the complex strips of muscle used in previous studies. The slow Ca current was evoked by stepping the membrane potential from a holding level negative to −50 mV, where channels are mainly closed, to various depolarized potentials where channels are opened. Flashes (1 ms) were presented to the cells at various times before the voltage step and during the depolarization. When flashes were delivered early during the step, the flash-induced current increased at a rate similar to the normal voltage jump-induced current, which was interpreted to reflect blockade of closed channels; removal of block allowed the channels to open at the normal rate. As found in previous studies (127, 137), almost complete recovery of I_{si} occurred when the flash was presented just prior to or with the voltage step. When the flash was presented later during the step, it became less effective at recovering I_{si}. A detailed analysis suggested that the time dependence of recovery reflected the appearance of inactivated channels that were bound by nifedipine. It was proposed that photoinactivation, although it removed nifedipine from the channels, did not induce recovery because the channels were unable to conduct. Complete recovery was observed after the membrane was repolarized, allowing the inactivated channels to return to the resting, closed state, ready for opening.[1]

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[1] These experiments also provide a simple explanation for earlier observations on the photoinduced recovery of action potential and twitch tension, recorded from frog ventricular muscle in the presence of nifedipine (127). Flashes of 200 ms delivered before the action potential effec-

Light-flash studies with nifedipine thus confirmed previous reports suggesting that nifedipine interacts with inactivated, as well as closed, Ca channels (7, 158). The technique additionally provided quantitative information about the mechanism of nifedipine antagonism that was not readily accessible by more conventional pharmacological approaches. Numerical simulations (70) of the effects of nifedipine on I_{si}, and of the effects of flashes presented at various times during I_{si} in the presence of nifedipine, provided good evidence that the data obtained from the light-flash experiments were well fit by the "modulated receptor" model of channel blockade (80, 82). Several other models have been developed to explain the effects of dihydropyridine Ca antagonists on cardiac Ca currents. For example, it was proposed recently (77) that Ca channels have several modes of gating and that transitions between modes occur on a time scale of seconds. Dihydropyridine Ca antagonists or agonists were subsequently proposed to stabilize channels in a particular mode (140). The rapid flash-induced recovery from nifedipine blockade shows that under certain conditions, these transitions occur much more rapidly.

B. Neurons

A similar study in the neurosecretory bag cells of the marine mollusc *Aplysia californica* suggested strongly that nifedipine blocks Ca channels in these cells in a similar manner to those in heart tissue (134). This study was much less detailed, however, because, although continuous illumination removed nifedipine blockade, single flashes were relatively ineffective. The reason for the lack of effect of flashes is unclear. Measurements of cell membrane capacitance provided values that were consistent with a smooth, spherical cell (A. M. Gurney and J. M. Nerbonne, unpublished data). Thus it is unlikely that nifedipine binds to channels within membrane infoldings, thereby being shielded from the light by a combination of an effectively thickened membrane and the intracellular orange-brown pigment present in these cells (58). The rapidity with which the small recovery from nifedipine blockade was induced by flashes does suggest, however, that most of the nifedipine-bound channels were in some way shielded from the light source. Although the intracellular pigment may possibly play some role, precisely what this role is has not been determined. One possibility is that the channels are localized in one area of the cell membrane and, in turn, this area is adjacent to the substrate to which the cells adhere on the bottom of the recording chamber (134). In this configuration, nifedipine-bound channels would be shielded from the light by the intracellular pigment (58). There are, however, no data at present to address this point. The problem may not only be restricted to Ca antagonist studies. Light flashes presented to these cells during exposure to 4,5-dimethoxy-2-

tively removed blockade, whereas light presented during the action potential had little effect. It is now clear that the later flashes were delivered while the Ca channels were inactivated; a brief repolarization was necessary to remove inactivation and reveal unblocked channels.

nitrobenzyl cAMP (caged cAMP, up to 200 μM) had no effects (J. M. Nerbonne and A. M. Gurney, unpublished data) on Ca currents and variable effects on potassium conductances, even though cAMP is known to have pronounced effects on potassium currents in these cells (90, 179). The study therefore emphasizes that not all cell types may be amenable to light-flash studies.

The small amount of unblocking of Ca currents that flashes did produce in the presence of nifedipine and nisoldipine nevertheless provided useful information for clarifying the mechanism of Ca antagonist action in bag cells (134). The responses to flashes showed qualitatively similar properties to those observed in cardiac muscle. Flash-induced currents increased rapidly, and the magnitude of unblocking was time dependent, flashes becoming less effective as they were presented later and later during the current waveform. Taken together with the qualitatively similar effects of the drug on the two cell types in the absence of flashes, this implies that similar mechanisms may well underlie Ca antagonism by nifedipine in these two different tissues. Nifedipine was, however, much less selective for Ca channels in this tissue. Higher drug concentrations were required to produce blockade comparable to that in heart, and the drug was only around threefold more effective at blocking Ca channels than potassium channels (134).

C. Calcium Agonists

Although we may soon understand how Ca antagonists produce their effects on Ca channels, we know much less about how the novel dihydropyridine Ca "agonists" (161) act to enhance the flow of current through Ca channels. Models have been proposed to explain the effects of both agonists and antagonists (7, 140, 159). As the dihydropyridine agonists apparently bind to the same site as the antagonists (134, 161), a single kinetic model might be expected to explain the actions of both types of drugs. In view of the information provided by light-flash experiments with antagonists, perhaps a photolabile agonist would be a useful tool for providing insights into Ca channel regulation that could not be obtained with more conventional approaches. The Ca agonist molecule BAY K 8644 does contain a nitro group, but not in an appropriate position for photolysis. Preliminary flash photolysis studies show no absorption changes on a millisecond time scale, although irradiation does produce slower spectral changes (J. M. Nerbonne, personal communication). Light flashes had little effect on Ca currents in *Aplysia* bag cells exposed to the agonist (J. M. Nerbonne and A. M. Gurney, unpublished data), and only slow changes in current have been observed in frog atrial trabeculae (J. Nargeot, personal communication). The search will therefore continue for a suitable light-sensitive agonist, and if found it will be used as a probe of Ca channel regulation and calcium agonist action.

IX. PROTONS

Several intracellular buffering systems ensure that cytoplasmic pH is tightly regulated (141, 156). The rates of virtually all enzyme reactions are

influenced by pH, so it is hardly surprising that pH changes that do occur have profound effects on many cellular properties (125, 141, 156). There is evidence that membrane ionic conductances are altered (reviewed in 125). Indeed protons may themselves act as charge carriers through specific proton channels in the plasma membrane (183). Intracellular pH also influences glycolysis (38, 184) and its stimulation in frog muscle by insulin (126), the secretion and reabsorption of acid across epithelial cells (16, 195, 196), and fertilization (49, 88, 165). Protons and Ca ions compete with each other for binding sites on a variety of molecules (22, 50), and changes in the intracellular concentration of either ion can influence the concentration of the other (2, 78, 107, 155). The increase in intracellular Ca^{2+} concentration caused by a rise in intracellular pH produces a greater force of contraction in cardiac Purkinje fibers (78), and similar mechanisms may contribute to comparable effects of intracellular pH on skeletal muscle tension (50).

In addition to modulating the properties of individual cells, intracellular pH also influences communication between groups of cells that are electrically and metabolically coupled (85, 176, 188). In the heart, smooth muscle, and some neurons, ionic coupling promotes synchronous electrical activity, resulting, in muscle, in synchronized contraction. In nonexcitable tissues, the passage of small molecules between cells could represent an important homeostatic mechanism and might regulate and synchronize various cellular activities such as differentiation, growth, and metabolism (116). In addition, coupling between cells may be involved in tissue responses to hormones and neurotransmitters (11, 104, 106, 116, 146, 181). This intercellular communicaton occurs via gap junctions (8). In many organisms, protons reduce the conductance of gap junction channels, thus inhibiting the passage of molecules through them (60, 85, 176, 188), although the precise mechanisms by which they do this is unknown.

Physiological techniques commonly used to change intracellular pH include direct internal perfusion of cells with buffers (21, 71, 192), exposing cells to weak acids such as CO_2 or acetate in the external solution, or exposing them for short periods to NH_4Cl in the external medium, which results in cytoplasmic acidification when normal medium is reintroduced (17). These methods are, however, far from ideal. To gain control over pH in internal perfusion experiments, very high concentrations of buffer are required (21). The effects of weak acids and NH_4Cl are unpredictable. They do not cause the same pH changes in all cells. In some cells the change is transient (46, 182), whereas in others it is prolonged (14). Another problem with these methods is that the pH changes occur slowly. Consequently, we know nothing about the time courses of the responses to pH changes and cannot distinguish clearly between direct and secondary effects of protons. It would obviously be desirable to have a method of changing intracellular pH in a controlled fashion that is rapid, reversible, and reproducible.

Nerbonne and colleagues (115, 133, 135, 136, 178) have taken a photochemical approach to developing physiological probes that will facilitate the study of responses to intracellularly applied protons and intracellular pH

buffering capacities. The systematic photochemical studies (summarized in sect. II*B*) suggest that *o*-nitrobenzyl esters with electron-donating substituents will be useful compounds for generating fast, efficient release of protons. Some molecules in this series are lipophilic and therefore equilibrate rapidly with the cell interior, avoiding the need for intracellular microinjection.

Photolabile proton donors have so far been used mainly to probe pH control of gap-junctional conductance (136, 178). Electrical coupling between cells of the salivary gland of the midge *Chironomus* were studied in experiments that involved either the whole gland or mechanically isolated pairs of glandular cells (136). When the preparations were bathed in *o*-nitrobenzylacetate, a single light flash caused a drop in junctional conductance, consistent with a fall in intracellular pH. Control experiments confirmed that the flash-induced modulation of junctional conductance was likely due to proton release inside the cells. Without irradiation, junctional conductance was unaffected by *o*-nitrobenzylacetate. In a number of other preparations, however, bath application of this ester, as well as other membrane-permeant nitrobenzylacetate derivatives, reduced gap-junctional conductance even in the dark (178). In millimolar concentrations, *o*-nitrobenzylacetate reduced junctional conductance between *Fundulus* embryonic cells (178), squid blastomeres (177), frog blastomeres (177), and in crayfish septate axon (177) within 1 min, with little effect on nonjunctional conductances. The decrease in conductance was well correlated with a fall in cytoplasmic pH, which was recorded spectrophotometrically after intracellular injection of phenol red in *Fundulus* cells (178) and with pH-sensitive microelectrodes in frog blastomeres and crayfish septate axon (177). It was concluded that the observed uncoupling resulted from a drop in intracellular pH that occurred in the absence of flashes. The suggestion is that, although in physiological salt solutions hydrolysis of nitrobenzyl esters is negligible, protons may be generated intracellularly as a result of cleavage of the ester by cytoplasmic esterases. This finding appears to vitiate the use of some permeant *o*-nitrobenzylacetates for flash-induced intracellular pH jumps; they still appear useful as nontoxic agents for blocking intercellular communication. They have several advantages over the more traditional methods of changing intracellular pH; they are effective at lower concentrations, have little effect on the conductance of nonjunctional membranes, and junctional conductance recovers even after prolonged exposures (177).

Photolabile proton donors have also been used to study the proton-driven motor of the bacterial flagellum (96). Streptococcal cells were exposed to 1 mM 2-hydroxyphenyl-1-(2-nitro)phenylethylphosphate, which is membrane impermeant. A single flash induced an extracellular pH jump of -0.73 units in <1 ms. Flashes increased the rate of rotation of the flagellum and indicated a lag in the response to proton jumps.

As well as providing a useful tool for studying the physiological effects of protons, photolabile proton donors are also essential for experiments in which the effects of other light-sensitive intracellular molecules are tested.

The close relationship between the intracellular concentrations of protons and Ca^{2+} was discussed earlier in this section. So it is clear that experiments designed to study the effects of changes in intracellular Ca^{2+} should also consider the possible effects of changes in cytoplasmic pH. Many of the photolabile molecules described in this review release a proton in response to irradiation in addition to the active molecule. The resulting cytoplasmic acidification should be considered in interpreting the data. Use of *o*-nitrobenzyl acetates in parallel experiments may provide an adequate control for changes in pH in some cells. It would clearly be more useful, however, to have a photolabile proton donor that is not a substrate for cytoplasmic esterases and could be used to provide proton jumps in all cell types. Esterase-resistant molecules suitable for flash-induced pH jumps are under development (J. M. Nerbonne, personal communication). Irie (84a) has also synthesized molecules suitable for flash-induced pH jumps in aqueous solutions.

X. COMMERCIAL SUPPORT

Many of the compounds discussed here are available from Molecular Probes, Eugene, OR. A suitable flashlamp system can be obtained from Chadwick-Helmuth, El Monte, CA.

XI. CONCLUSION

When light-flash experiments were last reviewed 5 years ago (110), the most detailed results had been obtained with nicotinic acetylcholine receptors. Since then, photosensitive molecules have begun to play important roles in explaining how intracellular messengers act. Within the past 2 years, both caged Ca and caged IP_3 have been developed and applied successfully (66, 70a, 80, 86, 200). The commercial support noted renders experiments more accessible for the physiological community. Even if *Polytechnus* never arrives, the next 5 years are certain to witness exciting activity in this field.

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REFERENCES

1. ADAMS, P. R., A. CONSTANTI, D. A. BROWN, AND R. B. CLARK. Intracellular Ca^{2+} activates a fast voltage-sensitive K^+ current in vertebrate sympathetic neurones. *Nature Lond.* 296: 746–749, 1982.

2. ALVAREZ-LEEFMANS, F. J., T. J. RINK, AND R. Y. TSIEN. Free calcium ions in neurones of *Helix aspersa* measured with ion-selective micro-electrodes. *J. Physiol. Lond.* 315: 531-548, 1981.
3. ATWATER, I., L. ROSARIO, AND E. ROJAS. Properties of the Ca-activated K^+ channel in pancreatic beta cells. *Cell Calcium* 4: 451-461, 1983.
4. BARRETT, J. N., K. L. MAGLEBY, AND B. S. PALLOTTA. Properties of single calcium-activated potassium channels in cultured rat muscle. *J. Physiol. Lond.* 331: 211-230, 1982.
5. BARTELS, E., N. H. WASSERMANN, AND B. F. ERLANGER. Photochromic activators of the acetylcholine receptor. *Proc. Natl. Acad. Sci. USA* 68: 1820-1823, 1971.
6. BARTELS-BERNAL, E., T. L. ROSENBERRY, AND H. W. CHANG. A membrane activation cycle induced by sulfhydryl reagents after affinity labeling of the acetylcholine receptor of electroplax. *Mol. Pharmacol.* 12: 813-819, 1976.
7. BEAN, B. P. Nitrendipine block of cardiac calcium channels: high affinity binding to the inactivated state. *Proc. Natl. Acad. Sci. USA* 81: 6388-6392, 1984.
8. BENNETT, M. V. L., AND D. C. SPRAY (Editors). *Gap Junctions.* Cold Spring Harbor, NY: Cold Spring Harbor, 1985.
9. BIETH, J., N. H. WASSERMANN, S. M. VRATSANOS, AND B. F. ERLANGER. Photoregulation of biological activity by photochromic agents. IV: A model for diurnal variation of enzymic activity. *Proc. Natl. Acad. Sci. USA* 66: 850-854, 1970.
10. BLACHE, D., J. NARGEOT, AND J. M. NERBONNE. Direct evidence for the inhibition of platelet aggregation and release by intracellular cyclic AMP produced with a new photoactivatable derivative. *Thromb. Haemostasis* 55: 168-172, 1986.
11. BLALOCK, J. E., AND J. D. STANTON. Common pathways of interferon and hormonal action. *Nature Lond.* 183: 406-408, 1980.
12. BLANK, M., L. M. SOO, N. H. WASSERMANN, AND B. F. ERLANGER. Photoregulated ion binding. *Science Wash. DC* 214: 70-72, 1981.
13. BLASIE, J. K., L. HERBETTE, D. PIERCE, D. PASCOLINI, A. SCARPA, AND S. FLEISCHER. Static and time-resolved structural studies of the Ca^{2+}-ATPase of isolated sarcoplasmic reticulum. *Ann. NY Acad. Sci.* 402: 478-484, 1982.
14. BOLTON, T. B., AND R. D. VAUGHAN-JONES. Continuous direct measurement of intracellular chloride and pH in frog skeletal muscle. *J. Physiol. Lond.* 270: 801-833, 1977.
15. BONINI, N. M., M. C. GUSTIN, AND D. L. NELSON. Regulation of ciliary motility by membrane potential in *Paramecium:* a role for cyclic AMP. *Cell Motil. Cytoskeleton* 6: 256-272, 1986.
16. BORON, W. F., AND E. L. BOULPAEP. Intracellular pH regulation in the salamander renal proximal tubule (abstr.). *Kidney Int.* 18: 126, 1980.
17. BORON, W. F., AND P. DE WEER. Intracellular pH transients in squid giant axons caused by CO_2, NH_3 and metabolic inhibitors. *J. Gen. Physiol.* 67: 91-112, 1976.
18. BREMEL, R. D., J. M. MURRAY, AND A. WEBER. Manifestations of cooperative behavior in the regulated actin filament during actin-activated ATP hydrolysis in the presence of calcium. *Cold Spring Harbor Symp. Quant. Biol.* 37: 267-275, 1973.
19. BREMEL, R. D., AND A. WEBER. Cooperation within actin filament in vertebrate skeletal muscle. *Nature New Biol.* 238: 97-101, 1972.
20. BYERLY, L., AND S. HAGIWARA. Calcium currents in internally perfused nerve cell bodies of *Limnea stagnalis.* *J. Physiol. Lond.* 322: 503-528, 1982.
21. BYERLY, L., AND W. J. MOODY. Intracellular pH changes in internally perfused snail neurons (abstr.). *Biophys. J.* 37: 181, 1982.
22. CARVALHO, A. F., H. SANUI, AND N. PACE. Calcium and magnesium binding properties of cell membrane materials. *J. Cell. Comp. Physiol.* 62: 311-317, 1963.
23. CASE, R. M. The role of Ca^{2+} stores in secretion. *Cell Calcium* 5: 89-110, 1984.
24. CAUVIN, C., R. LOUTZENHISER, AND C. VAN BREEMEN. Mechanisms of calcium antagonist-induced vasodilation. *Annu. Rev. Pharmacol. Toxicol.* 23: 373-396, 1983.
25. CAVERO, I., AND M. SPEDDING. "Calcium antagonists": a class of drugs with a bright future. I. Cellular calcium homeostasis and calcium as a coupling messenger. *Life Sci.* 33: 2571-2581, 1983.
26. CHABALA, L. D., A. M. GURNEY, AND H. A. LESTER. Dose-response of acetylcholine receptor channels opened by a flash-activated agonist in voltage-clamped rat myoballs. *J. Physiol. Lond.* 371: 407-433, 1985.
27. CHABALA, L. D., A. M. GURNEY, AND H. A. LESTER. Photoactivation and dissociation of agonist molecules at the nicotinic acetylcholine receptor in voltage-clamped rat myoballs. *Biophys. J.* 48: 241-246, 1985.
28. CHABALA, L. D., AND H. A. LESTER. Voltage dependence of acetylcholine receptor channel opening in rat myoballs (abstr.). *J. Gen. Physiol.* 84: 24, 1984.
29. CHABALA, L. D., AND H. A. LESTER. Kinetic and steady-state properties of acetylcholine receptor channels in voltage-clamped rat myoballs (abstr.). *Biophys. J.* 47: 257, 1985.
30. CHABALA, L. D., AND H. A. LESTER. Activation of acetylcholine receptor channels by covalently bound agonists in cultured rat myoballs. *J. Physiol Lond.* 379: 83-108, 1986.
31. COBBS, W. H., AND E. N. PUGH, JR. Cyclic GMP can increase rod outer-segment light-sensitive current 10-fold without delay of excitation. *Nature Lond.* 313: 585-587, 1985.
32. COLQUHOUN, D., E. NEHER, H. REUTER, AND C. F. STEVENS. Inward current channels activated by intracellular Ca in cultured cardiac cells. *Nature Lond.* 294: 752-754, 1981.
33. COLQUHOUN, D., AND B. SAKMANN. Fast events in single channel currents activated by acetylcholine and its analogues at the frog muscle end-plate. *J. Physiol. Lond.* 369: 501-557, 1985.
34. CURTIS, B. M., AND W. A. CATTERALL. Solubilization of the calcium antagonist receptor from rat brain. *J. Biol. Chem.* 258: 7280-7283, 1983.
35. CURTIS, B. M., AND W. A. CATTERALL. Purification of the calcium antagonist receptor of the voltage-sensitive calcium channel from skeletal muscle transverse tubules. *Biochemistry* 23: 2113-2118, 1984.
36. CURTIS, B. M., AND W. A. CATTERALL. Phosphorylation of the calcium antagonist receptor of the voltage-sensitive calcium channel by cAMP-dependent protein kinase. *Proc. Natl. Acad. Sci. USA* 82: 2528-2532, 1985.
37. CUTHBERT, A. W., AND J. A. SPAYNE. Stimulation of sodium and of chloride transport in epithelia by forskolin. *Br. J. Pharmacol.* 76: 33-35, 1982.
38. DANFORTH, W. H. Activation of glycolytic pathway in muscle. In: *Control of Energy Metabolism,* edited by B. Chance, R. W. Estabrook, and J. B. Williamson. New York: Academic, 1965, p. 287-298.
39. DANTZIG, J. A., M. G. HIBBERD, Y. E. GOLDMAN, AND D. R. TRENTHAM. ADP slows cross-bridge detachment

rate induced by photolysis of caged ATP in rabbit psoas muscle-fibers (abstr.). *Biophys. J.* 45: 8, 1984.

40. DANTZIG, J. A., D. R. TRENTHAM, AND Y. E. GOLDMAN. Mechanics of muscle contraction and relaxation in ϵ-ATP and caged ϵ-ATP (abstr.). *Biophys. J.* 47: 25, 1985.
41. DELCOUR, A., D. J. CASH, B. F. ERLANGER, AND G. P. HESS. Separation of *cis* and *trans*-3,3'-bis-(trimethylammoniomethyl)azobenzene salts (Bis-Q salts) by reversed-phase high-performance liquid chromatography. *J. Chromatogr.* 248: 461-468, 1982.
42. DELCOUR, A., AND G. P. HESS. Chemical kinetic measurements of the effect of *trans* and *cis* Bis[(trimethylammonio)methyl]azobenzene bromide on acetylcholine receptor mediated ion translocation in *Electrophorus electricus* and *Torpedo californica*. *Biochemistry* 25: 1793-1798, 1986.
43. DOUGLAS, W. W., AND R. P. RUBIN. The role of calcium in the secretory response of the adrenal medulla to acetylcholine. *J. Physiol. Lond.* 159: 40-57, 1961.
44. EBASHI, S. Ca^{2+} in biological systems. *Experientia Basel* 41: 978-981 1985.

44a. EBEL, V. S., H. SCHUTZ, AND A. HORNITSCHEK. Untersuchungen zur Analytik von Nifedipin unter besonderer Berücksichtigung der bei lichtexposition entstehenden Umwandlungsprodukte. *Arzneim. Forsch.* 28: 2188-2193, 1978.

45. ECKERT, R., AND D. TILLOTSON. Potassium activation associted with intraneuronal free calcium. *Science Wash. DC* 200: 437-439, 1978.
46. ELLIS, D., AND R. C. THOMAS. Direct measurement of the intracellular pH of mammalian cardiac muscle. *J. Physiol. Lond.* 262: 755-771, 1976.
47. ENGELS, J., AND R. REIDYS. Synthesis and application of the photolabile guanosine 3',5'-phosphoric-*o*-nitrobenzylester. *Experentia Basel* 34: 14-15, 1978.
48. ENGELS, J., AND E.-J. SCHLAEGER. Synthesis, structure and reactivity of adenosine cyclic 3', 5'-phosphate benzyl triesters. *J. Med. Chem.* 20: 907-911, 1977.
49. EPEL, D., R. STEINHARDT, T. HUMPHREYS, AND D. MAZIA. An analysis of the partial metabolic depression of sea urchin eggs by ammonia; the existence of independent pathways. *Dev. Biol.* 40: 245-255, 1974.
50. FABIATO, A., AND F. FABIATO. Effects of pH on the myofilaments and the sarcoplasmic reticulum of skinned cells from cardiac and skeletal muscle. *J. Physiol. Lond.* 276: 233-255, 1978.
51. FENWICK, E. M., A. MARTY, AND E. NEHER. Sodium and calcium channels in bovine chromaffin cells. *J. Physiol. Lond.* 331: 599-635, 1982.
52. FESENKO, E. E., S. S. KOLESNIKOV, AND A. L. LYUBARSKY. Induction by cyclic GMP of cationic conductance in plasma membrane of retinal rod outer segments. *Nature Lond.* 313: 310-313, 1985.
53. FLECKENSTEIN, A. Specific inhibitors and promotors of calcium action in the excitation-contraction coupling of heart muscle and their role in the production or prevention of myocardial lesions. In: *Calcium and the Heart*, edited by P. Harris and L. Opie. New York: Academic, 1971, p. 135-188.
54. FLECKENSTEIN, A. History of calcium antagonists. *Circ. Res.* 53, *Suppl.* 1: 3-16, 1983.
55. FLECKENSTEIN, A., H. TRITTHART, H. J. DÖRING, AND Y. K. BYON. Bay a 1040—ein hochaktiver Ca^{++}-antagonistischer Inhibitor der elektromechanischen Koppelungsprozesse im Warmblüter-Myokard. *Arzneim. Forsch.* 22: 22-33, 1972.
56. FORBUSH, B. Na^+ movement in a single turnover of the Na pump. *Proc. Natl. Acad. Sci. USA* 81: 5310-5314, 1984.
57. FOSSET, M., E. JAIMOVICH, E. DELPONT, AND M. LAZDUNSKI. [^{3}H]nitrendipine receptors in skeletal muscle. *J. Biol. Chem.* 258: 6086-6092, 1983.
58. FRAZIER, W. T., E. R. KANDEL, I. KUPFERMANN, R. WAZIRI, AND R. E. COGGESHALL. Morphological and functional properties of identified neurons in the abdominal ganglion of *Aplysia californica*. *J. Neurophysiol.* 30: 1288-1351, 1967.
59. GARNIER, D., H. LESTER, J. NARGEOT, J. NERBONNE, AND S. RICHARD. Excitation-contraction coupling in frog atrial muscle during intracellular jumps of cAMP (abstr.). *J. Physiol. Lond.* 345: 30P, 1983.
60. GIAUME, C., M. E. SPIRA, AND H. KORN. Uncoupling of invertebrate electrotonic synapses by carbon dioxide. *Neurosci. Lett.* 17: 197-202, 1980.
61. GLITSCH, H. G., AND L. POTT. Effects of acetylcholine in parasympathetic nerve stimulation on membrane potential in quiescent guinea-pig atria. *J. Physiol. Lond.* 279: 655-668, 1978.
62. GLOSSMAN, H., D. R. FERRY, AND C. B. BOSCHEK. Purification of the putative calcium channel from skeletal muscle with the aid of [^{3}H]nimodipine binding. *Naunyn-Schmiedebergs Arch. Pharmakol.* 323: 1-11, 1983.
63. GOLDMAN, Y. E., M. G. HIBBERD, J. A. MCCRAY, AND D. R. TRENTHAM. Relaxation of muscle fibres by photolysis of caged ATP. *Nature Lond.* 300: 701-705, 1982.
64. GOLDMAN, Y. E., M. G. HIBBERD, AND D. R. TRENTHAM. Relaxation of rabbit psoas muscle fibres from rigor by photochemical generation of adenosine-5'-triphosphate. *J. Physiol. Lond.* 354: 577-604, 1984.
65. GOLDMAN, Y. E., M. G. HIBBERD, AND D. R. TRENTHAM. Initiation of active contraction by photogeneration of adenosine-5'-triphosphate in rabbit psoas muscle fibres. *J. Physiol. Lond.* 354: 605-624, 1984.
66. GOLDMAN, Y. E., G. P. REID, A. P. SOMLYO, A. V. SOMLYO, D. R. TRENTHAM, AND J. W. WALKER. Activation of skinned vascular smooth muscle by photolysis of "caged inositol trisphosphate" to inositol 1,4,5-trisphosphate ($InsP_3$). *J. Physiol. Lond.* 377: 100P, 1986.
67. GOODY, R. S., K. GÜTH, Y. MAÈDA, K. J. V. POOLE, AND G. RAPP. Time-resolved X-ray diffraction measurements on *Lethocerus* fibrillar flight muscle following the photolytic release of ATP from "caged-ATP" (abstr.). *J. Physiol. Lond.* 364: 75P, 1985.
68. GOULD, R. J., K. M. M. MURPHY, AND S. H. SNYDER. ^{3}H-nitrendipine labeled channels discriminate inorganic calcium agonists and antagonists. *Proc. Natl. Acad. Sci. USA* 79: 3656-3660, 1982.
69. GURNEY, A. J., J. NARGEOT, H. A. LESTER, S. ADAMS, J. KAO, AND R. Y. TSIEN. Potassium channels in rat sympathetic neurons activated by flash-induced intracellular Ca^{2+} jumps. *Soc. Neurosci. Abstr.* 12: 560, 1986.
70. GURNEY, A. M., J. M. NERBONNE, AND H. A. LESTER. Photoinduced removal of nifedipine reveals mechanisms of calcium antagonist action on single heart cells. *J. Gen. Physiol.* 86: 353-379, 1985.

70a. GURNEY, A. M., R. Y. TSIEN, AND H. A. LESTER. Activation of a potassium current by rapid photochemically generated steps of intracellular calcium in rat sympathetic neurons. *Proc. Natl. Acad. Sci. USA*. In press.

71. HAGIWARA, S., R. GRUENER, H. HAYASHI, H. SAKATA, AND A. D. GRINNELL. Effect of external and internal pH changes on K and Cl conductances in the muscle fiber membrane of a giant barnacle. *J. Gen. Physiol.* 52: 773-792, 1968.

72. HAMILL, O. P., A. MARTY, E. NEHER, B. SAKMANN, AND F. J. SIGWORTH. Improved patch-clamp techniques for high-resolution current recording from cells and cell-free membrane patches. *Pfluegers Arch.* 391: 85–100, 1981.
73. HANSFORD, R. G., AND J. B. CHAPPELL. The effect of Ca^{2+} on the oxidation of glycerophosphate by blowfly flight-muscle mitochondria. *Biochem. Biophys. Res. Commun.* 27: 686–692, 1967.
74. HARTZELL, H. C. Distribution of muscarinic receptors and presynaptic nerve terminals in amphibian heart. *J. Cell Biol.* 86: 6–20, 1980.
74a. HARTZELL, H. C., AND R. FISCHMEISTER. Opposite effects of cyclic GMP and cyclic AMP on Ca^{2+} current in single heart cells. *Nature Lond.* 323: 273–275, 1986.
74b. HAYNES, L., AND K. W. YAU. Cyclic GMP-sensitive conductance in outer segment membrane of catfish cones. *Nature Lond.* 317: 61–64, 1985.
75. HENRY, P. D. Comparative pharmacology of calcium antagonists: nifedipine, verapamil and diltiazem. *Am. J. Cardiol.* 46: 1047–1058, 1980.
76. HERBETTE, L., AND J. K. BLASIE. Static and time-resolved structural studies on isolated sarcoplasmic reticulum membranes. In: *Calcium Binding Proteins*, edited by F. L. Siegel, E. Carafoli, R. H. Kretsinger, D. H. Maclennan, and R. H. Wassermann. Amsterdam: Elsevier/North-Holland, 1980, p. 115–120.
77. HESS, P., J. G. LANSMAN, AND R. W. TSIEN. Different modes of Ca channel gating behaviour favoured by dihydropyridine Ca agonists and antagonists. *Nature Lond.* 311: 538–544, 1984.
78. HESS, P., AND R. WEINGART. Intracellular free calcium modified by pH_i in sheep cardiac Purkinje fibres. *J. Physiol. Lond.* 307: 60P–61P, 1980.
79. HIBBERD, M. S., J. A. DANTZIG, D. R. TRENTHAM, AND Y. E. GOLDMAN. Phosphate release and force generation in skeletal muscle fibers. *Science Wash. DC* 228: 1317–1319, 1981.
80. HILLE, B. Local anesthetics: hydrophilic and hydrophobic pathways for the drug-receptor interaction. *J. Gen. Physiol.* 69: 497–515, 1977.
81. HILL-SMITH, I., AND R. D. PURVES. Synaptic delay in the heart: an ionophoretic study. *J. Physiol. Lond.* 279: 31–54, 1978.
82. HONDEGHEM, L. M., AND B. G. KATZUNG. Time- and voltage-dependent interactions of antiarrhythmic drugs with cardiac sodium channels. *Biochim. Biophys. Acta* 472: 373–398, 1977.
83. HONDEGHEM, L. M., AND B. G. KATZUNG. Antiarrhythmic agents: the modulated receptor mechanism of action of sodium and calcium channel-blocking drugs. *Annu. Rev. Pharmacol. Toxicol.* 24: 387–423, 1984.
84. HUME, J. R. Comparative interactions of organic Ca^{++} channel antagonists with myocardial Ca^{++} and K^+ channels. *J. Pharmacol. Exp. Ther.* 234: 134–140, 1985.
84a. IRIE, M. Light-induced reversible pH change. *J. Am. Chem. Soc.* 105: 2078–2079, 1983
85. IWATSUKI, N., AND O. H. PETERSON. Pancreatic acinar cells: the effect of carbon dioxide, ammonium chloride and acetylcholine on intercellular communication. *J. Physiol. Lond.* 291: 317–326, 1979.
86. JANIS, R. A., AND D. J. TRIGGLE. New developments in Ca^{++} channel antagonists. *J. Med. Chem.* 26: 775–785, 1983.
87. JENKINSON, D. H., D. G. HAYLETT, AND N. S. COOK. Calcium-activated potassium channels in liver cells. *Cell Calcium* 4: 429–437, 1983.
88. JOHNSON, J. J., D. EPEL, AND M. PAUL. Intracellular pH and activation of sea urchin eggs after fertilisation. *Nature Lond.* 262: 661–664, 1976.
89. KACZMAREK, L. K., AND F. STRUMWASSER. The expression of long-lasting afterdischarge by isolated *Aplysia* bag cell neurons. *J. Neurosci.* 1: 626–634, 1981.
90. KACZMAREK, L. K., AND F. STRUMWASSER. A voltage-clamp analysis of currents underlying cyclic AMP-induced membrane modulation in isolated peptidergic neurons of *Aplysia*. *J. Neurophysiol.* 52: 340–349, 1984.
91. KANO, K., Y. TANAKA, T. OGAWA, M. SHIMOMURA, AND T. KUNITAKE. Photoresponsive artificial membrane. Regulation of membrane permeability of liposomal membrane by photoreversible *cis-trans* isomerization of azobenzenes. *Photochem. Photobiol.* 34: 323–329, 1981.
91a. KANO, K., Y. TANAKA, T. OGAWA, M. SHIMOMURA, AND T. KUNITAKE. Photoresponsive membranes. Regulation of membrane properties by photoreversible *cis-trans* isomerization of azobenzenes. *Chem. Lett.* 421–424, 1980.
92. KAPLAN, J. H. Sodium pump-mediated ATP:ADP exchange. The sided effects of sodium and potassium ions. *J. Gen. Physiol.* 80: 915–937, 1982.
93. KAPLAN, J. H., B. FORBUSH, AND J. F. HOFFMAN. Rapid photolytic release of adenosine 5′-triphosphate from a protected analogue: utilization by the Na:K pump of human red blood cell ghosts. *Biochemistry* 17: 1929–1935, 1978.
94. KAPLAN, J. H., AND R. J. HOLLIS. External Na dependence of ouabain-sensitive ATP:ADP exchange initiated by photolysis of intracellular caged-ATP in human red cells. *Nature Lond.* 288: 587–589, 1980.
95. KATZ, B., AND R. MILEDI. A study of synaptic transmission in the absence of nerve impulses. *J. Physiol. Lond.* 192: 407–436, 1967.
96. KHAN, S., M. MEISTER, AND H. C. BERG. Constraints on flagellar rotation. *J. Mol. Biol.* 184: 645–656, 1985.
97. KLEIN, M., J. S. CAMARDO, AND E. R. KANDEL. Serotonin modulates a specific potassium current in the sensory neurones that show presynaptic facilitation in *Aplysia*. *Proc. Natl. Acad. Sci. USA* 79: 5713–5717, 1982.
98. KORTH, M., AND J. ENGELS. The effects of adenosine- and guanosine 3′,5′-phosphoric acid benzyl esters on guinea-pig ventricular myocardium. *Naunyn-Schmiedebergs Arch. Pharmakol.* 310: 103–111, 1979.
99. KOSTYUK, P. G. Calcium channels in the neuronal membrane. *Biochim. Biophys. Acta* 650: 128–150, 1981.
100. KROUSE, M. E., H. A. LESTER, M. M. NASS, J. M. NERBONNE, N. H. WASSERMANN, AND B. F. ERLANGER. ACh receptors begin to open within 10 μsec after agonist is applied. *Soc. Neurosci. Abstr.* 5: 483, 1979.
101. KROUSE, M. E., H. A. LESTER, N. H. WASSERMAN, AND B. F. ERLANGER. Rates and equilibria for a photoisomerizable antagonist at the acetylcholine receptor of *Electrophorus* electroplaques. *J. Gen. Physiol.* 85: 235–256, 1985.
102. KROUSE, M. E., H. A. LESTER, AND M. M. WEINSTOCK. How photoisomerizable azobenzene compounds affect acetylcholine receptors of skate muscle (abstr.). *Biol. Bull. Woods Hole* 157: 376, 1979.
103. LABARCA, P., M. S. MONTAL, J. LINDSTROM, AND M. MONTAL. The occurrence of long openings in the purified cholinergic channel increases with acetylcholine concentration. *J. Neurosci.* 12: 3409–3413, 1985.
104. LASATER, E. M., AND J. E. DOWLING. Dopamine decreases conductance of the electrical junctions between cultured retinal horizontal cells. *Proc. Natl. Acad. Sci. USA* 82: 3025–3029, 1985.

105. LATOREE, R., C. VERGARA, AND E. MOCZYDLOWSKI. Properties of a Ca^{2+}-activated K^+ channel in a reconstituted system. *Cell Calcium* 4: 343-357, 1983.
106. LAWRENCE, T. S., W. H. BEERS, AND N. B. GILULA. Transmission of hormonal stimulation by cell-to-cell communication. *Nature Lond.* 272: 501-506, 1978.
107. LEA, T. J., AND C. C. ASHLEY. Increase in free Ca^{2+} in muscle after exposure to CO_2. *Nature Lond.* 275: 236-238, 1978.
108. LEE, K. S., AND R. W. TSIEN. Mechanism of calcium channel blockade by verapamil, D600, diltiazem and nitrendipine in single dialyzed heart cells. *Nature Lond.* 302: 790-794, 1983.
109. LESTER, H. A., L. D. CHABALA, A. M. GURNEY, AND R. E. SHERIDAN. Experiments with photoisomerizable molecules at nicotinic acetylcholine receptors in cells and membrane patches from rat muscle. In: *Optical Methods in Cell Physiology*, edited by P. De Weer and B. Salzberg. New York: Wiley, 1986, vol. 40, p. 447-462. (Soc. Gen. Physiol. Ser.)
110. LESTER, H. A., AND H. W. CHANG. Response of acetylcholine receptors to rapid, photochemically produced increases in agonist concentration. *Nature Lond.* 266: 373-374, 1977.
111. LESTER, H. A., D. D. KOBLIN, AND R. E. SHERIDAN. Role of voltage-sensitive receptors in nicotinic transmission. *Biophys. J.* 21: 181-194, 1978.
112. LESTER, H. A., M. E. KROUSE, M. M. NASS, N. H. WASSERMANN, AND B. F. ERLANGER. Light-activated drug confirms a mechanism of ion channel blockade. *Nature Lond.* 280: 509-510, 1979.
113. LESTER, H. A., M. E. KROUSE, M. M. NASS, N. H. WASSERMANN, AND B. F. ERLANGER. A covalently bound photoisomerizable agonist. Comparison with reversibly bound agonists at *Electrophorus* electroplaques. *J. Gen. Physiol.* 75: 207-232, 1980.
114. LESTER, H. A., M. M. NASS, M. E. KROUSE, J. M. NERBONNE, N. H. WASSERMANN, AND B. F. ERLANGER. Electrophysiological experiments with photoisomerisable cholinergic compounds. Review and Progress Report. *Ann. NY Acad. Sci.* 346: 475-490, 1980.
115. LESTER, H. A., AND J. M. NERBONNE. Physiological and pharmacological manipulations with light flashes. *Annu. Rev. Biophys. Bioeng.* 11: 151-175, 1982.
116. LOEWENSTEIN, W. R. Junctional intercellular communication: the cell-to-cell membrane channel. *Physiol. Rev.* 61: 829-913, 1981.
117. MARANGOS, P. J., J. PATEL, C. MILLER, AND A. M. MARTINO. Specific calcium antagonist binding sites in brain. *Life Sci.* 31: 1575-1585, 1982.
118. MARUYAMA, Y., AND O. H. PETERSEN. Voltage clamp study of stimulant-evoked currents in mouse pancreatic acinar cells. *Pfluegers Arch.* 399: 54-62, 1983.
119. MATHEWS, H. R., V. TORRE, AND T. D. LAMB. Effects on the photoresponse of calcium buffers and cyclic GMP incorporated into the cytoplasm of retinal rods. *Nature Lond.* 313: 582-585, 1985.
120. MCCORMACK, J. G., AND R. M. DENTON. The effects of calcium ions and adenine nucleotides on the activity of pig heart 2-oxoglutarate dehydrogenase complex. *Biochem. J.* 180: 533-544, 1979.
121. MCCRAY, J. A., L. HERBETTE, T. KIHARA, AND D. R. TRENTHAM. A new approach to time-resolved studies of ATP-requiring biological systems: laser flash photolysis of caged ATP. *Proc. Natl. Acad. Sci. USA* 77: 7237-7241, 1980.
122. MEECH, R. W. Calcium-dependent potassium activation in nervous tissues. *Annu. Rev. Biophys. Bioeng.* 7: 1-18, 1978.
123. MILLER, R. J., AND S. B. FREEDMAN. Are dihydropyridine binding sites voltage sensitive calcium channels? *Life Sci.* 34: 1205-1221, 1984.
124. MILLER, W. H., AND G. D. NICOL. Evidence that cyclic GMP regulates membrane potential in rod photoreceptors. *Nature Lond.* 280: 64-66, 1979.
125. MOODY, W. Effects of intracellular H^+ on the electrical properties of excitable cells. *Annu. Rev. Neurosci.* 7: 257-278, 1984.
126. MOORE, R. D., M. L. FIDELMAN, J. C. HANSEN, AND J. N. OTIS. The role of intracellular pH in insulin action. In: *Intracellular pH: Its Measurement, Regulation and Utilisation in Cellular Functions*, edited by R. Nuccitelli and D. Deamer. New York: Liss, 1982.
127. MORAD, M., Y. E. GOLDMAN, AND D. R. TRENTHAM. Rapid photochemical inactivation of Ca^{2+}-antagonists shows that Ca^{2+}-entry directly activates contraction in frog heart. *Nature Lond.* 304: 635-638, 1983.
128. NAMM, D. H., AND S. E. MAYER. Effects of epinephrine on cardiac cyclic 3',5'-AMP, phosphorylase kinase, and phosphorylase. *Mol. Pharmacol.* 4: 61-69, 1968.
129. NARGEOT, J., H. A. LESTER, N. J. M. BIRDSALL, J. STOCKTON, N. H. WASSERMANN, AND B. F. ERLANGER. A photoisomerizable muscarinic antagonist: studies of binding and of conductance relaxations in frog heart. *J. Gen. Physiol.* 79: 657-678, 1982.
130. NARGEOT, J., J. M. NERBONNE, J. ENGELS, AND H. A. LESTER. Time course of the increase in the myocardial slow inward current after a photochemically generated concentration jump of intracellular cAMP. *Proc. Natl. Acad. Sci. USA* 80: 2395-2399, 1983.
131. NASS, M. M., H. A. LESTER, AND M. E. KROUSE. Response of acetylcholine receptors to photoisomerizations of bound agonist molecules. *Biophys. J.* 24: 135-160, 1978.
132. NAYLER, W. G. The heterogeneity of the slow channel blockers (calcium antagonists). *Int. J. Cardiol.* 3: 391-400, 1983.
133. NERBONNE, J. M. Design and application of photolabile intracellular probes. In: *Optical Methods in Cell Physiology*, edited by P. DeWeer and B. Salzberg. New York: Wiley, 1986, vol. 40, p. 417-446. (Soc. Gen. Physiol. Ser.)
134. NERBONNE, J. M., AND A. M. GURNEY. Ca^{++} antagonists block neuronal Ca^{++} and K^+ currents. *J. Neurosci.* In press.
135. NERBONNE, J. M., AND H. A. LESTER. The design of photoactivated molecules: chemical and structural control of reaction rates and efficiencies (abstr.). *Biophys. J.* 41: 3, 1983.
136. NERBONNE, J. M., H. A. LESTER, AND J. A. CONNOR. Photolabile proton donors and pH control of gap junctions. *Soc. Neurosci. Abstr.* 8: 945, 1982.
137. NERBONNE, J. M., S. RICHARD, AND J. NARGEOT. Ca^{++} channels are unblocked within a few msec after photoconversion of nifedipine. *J. Mol. Cell. Cardiol.* 17: 511-515, 1985.
138. NERBONNE, J. M., S. RICHARD, J. NARGEOT, AND H. A. LESTER. New photoactivatable cyclic nucleotides produce intracellular jumps in cyclic AMP and cyclic GMP concentrations. *Nature Lond.* 310: 74-76, 1984.
139. NERBONNE, J. M., R. E. SHERIDAN, L. D. CHABALA, AND H. A. LESTER. *Cis*-3,3'-Bis-[alpha-trimethylammonium)methyl]azobenzene (*cis*-Bis-Q): purification and properties at acetylcholine receptors of *Electrophorus* electroplaques. *Mol. Pharmacol.* 23: 344-349, 1983.
140. NOWYCKY, M. C., A. P. FOX, AND R. W. TSIEN. Long-opening mode of gating of neuronal calcium channels and

its promotion by the dihydropyridine calcium agonist Bay K 8644. *Proc. Natl. Acad. Sci. USA* 82: 2178–2182, 1985.
141. NUCCITELLI, R., AND D. DEAMER (Editors). *Intracellular pH: Its Measurement, Regulation and Utilization in Cellular Functions.* New York: Liss, 1982.
142. OPIE, L. H. (Editor). *Calcium Antagonists and Cardiovascular Disease.* New York: Raven, 1984.
143. OZAWA, E. Activation of phosphorylase kinase from brain by small amounts of calcium ion. *J. Neurochem.* 20: 1487–1488, 1973.
144. OZAWA, E., K. HOSOI, AND S. EBASHI. Reversible stimulation of muscle phosphorylase kinase by low concentration of calcium ions. *J. Biochem. Tokyo* 61: 531–533, 1967.
145. PALLOTTA, B. S., K. L. MAGLEBY, AND J. N. BARRETT. Single-channel recordings of Ca^{2+}-activated K^+ currents in rat muscle cell culture. *Nature Lond.* 293: 471–474, 1981.
146. PICCOLINO, M., J. NEYTON, P. WITKOWSKY, AND H. M. GERSCHENFELD. Gamma-aminobutyric acid antagonists decrease junctional communication between L-horizontal cells of the retina. *Proc. Natl. Acad. Sci. USA* 79: 3671–3675, 1982.
147. PIERCE, D. H., A. SCARPA, M. R. TOPP, AND J. K. BLASIE. Kinetics of calcium uptake by isolated sarcoplasmic reticulum vesicles using flash photolysis of caged adenosine 5'-triphosphate. *Biochemistry* 22: 5254–5261, 1983.
148. PIERCE, D. H., A. SCARPA, D. R. TRENTHAM, M. R. TOPP, AND J. K. BLASIE. Comparison of the kinetics of calcium transport in vesicular dispersions and oriented multilayers of isolated sarcoplasmic reticulum membranes. *Biophys. J.* 44: 365–373, 1983.
149. RAPOPORT, R. M., S. A. WALDMAN, K. SCHWARTZ, R. J. WINQUIST, AND F. MURAD. Effects of atrial natriuretic factor, sodium nitroprusside, and acetylcholine on cyclic GMP levels and relaxation in rat aorta. *Eur. J. Pharmacol.* 115: 219–229, 1985.
150. RASMUSSEN, H., AND P. Q. BARRETT. Calcium messenger system: an integrated view. *Physiol. Rev.* 64: 938–984, 1984.
151. RENGASAMY, A., J. PTASIENSKI, AND M. M. HOSEY. Purification of the cardiac 1,4-dihydropyridine receptor/calcium channel complex. *Biochem. Biophys. Res. Commun.* 126: 1–7, 1985.
152. REUTER, H. The dependence of slow inward current in Purkinje fibres on the extracellular calcium-concentration. *J. Physiol. Lond.* 192: 479–492, 1967.
153. RICHARD, S., J. M. NERBONNE, J. NARGEOT, H. A. LESTER, AND D. GARNIER. Photochemically produced intracellular concentration jumps of cAMP mimic the effects of catecholamines on excitation contraction coupling in frog atrial fibers. *Pfluegers Arch.* 403: 312–317, 1985.
154. RINGER, S., AND D. W. BUXTON. Upon the similarity and dissimilarity of the behavior of cardiac and skeletal muscle when brought into relation with solutions containing sodium, calcium, and potassium salts. *J. Physiol. Lond.* 8: 288–295, 1887.
155. RINK, T. J., R. Y. TSIEN, AND A. E. WARNER. Free calcium in *Xenopus* embryos measured with ion-selective microelectrodes. *Nature Lond.* 283: 658–660, 1980.
156. ROOS, A., AND W. F. BORON. Intracellular pH. *Physiol. Rev.* 61: 296–434, 1981.
157. SAKMANN, B., AND E. NEHER (Editors). *Single-Channel Recording.* New York: Plenum, 1983.
158. SANGUINETTI, M. C., AND R. S. KASS. Voltage-dependent block of calcium channel current in the calf cardiac Purkinje fiber by dihydropyridine calcium channel antagonists. *Circ. Res.* 55: 336–348, 1984.
159. SANGUINETTI, M. C., AND R. S. KASS. Photoalteration of calcium channel blockade in the cardiac Purkinje fiber. *Biophys. J.* 45: 873–880, 1984.
160. SCHLOSSMANN, K. Fluorometrische Bestimmung des 4-(2'-Nitrophenyl)-2,6-dimethyl-1,4-dihydropyridin-3,5-dicarbonsäuredimethylester und seines Hauptmetaboliten. *Arzneim. Forsch.* 22: 60–62, 1972.
161. SCHRAMM, M., G. THOMAS, R. TOWART, AND G. FRANCKOWIAK. Novel dihydropyridines with positive inotropic action through activation of Ca^{2+} channels. *Nature Lond.* 303: 535–537, 1983.
162. SCHRAMM, M., AND R. TOWART. Modulation of calcium channel function by drugs. *Life Sci.* 37: 1843–1860, 1985.
163. SCHWARTZ, A., AND D. J. TRIGGLE. Cellular action of calcium channel blocking drugs. *Annu. Rev. Med.* 35: 325–339, 1984.
164. SCHWARTZ, L. M., E. W. MCCLESKEY, AND W. ALMERS. Dihydropyridine receptors in muscle are voltage-dependent but most are not functional calcium channels. *Nature Lond.* 314: 747–751, 1985.
165. SHEN, S., AND R. A. STEINHARDT. Intracellular pH controls the development of new potassium conductance after fertilization of the sea urchin egg. *Exp. Cell Res.* 125: 55–61, 1980.
166. SHERIDAN, R. E., AND H. A. LESTER. Functional stoichiometry at the nicotinic receptor. The photon cross-section for phase I corresponds to two Bis-Q molecules per channel. *J. Gen. Physiol.* 80: 499–515, 1982.
167. SHINKAI, S., Y. HONDA, Y. KUSANO, AND O. MANABE. A photoresponsive cylindrical ionophore. *J. Chem. Soc. Chem. Commun.* 848–850, 1982.
168. SHINKAI, S., Y. HONDA, K. UEDA, AND O. MANABE. Photoresponsive crown ethers. Part 11. Azobenzene-pillared cylindrical macrocycle as a photoresponsive receptor. *Isr. J. Chem.* 24: 302–306, 1984.
169. SHINKAI, S., AND O. MANABE. Photocontrol of ion extraction and ion transport by photofunctional crown ethers. *Top. Curr. Chem.* 121: 67–104, 1984.
170. SHINKAI, S., K. MATSUO, A. HARADA, AND O. MANABE. Photocontrol of micellar catalyses. *J. Chem. Soc. Perkin Trans.* 2: 1261–1265, 1982.
171. SHINKAI, S., K. SHIGEMATSU, Y. HONDA, AND O. MANABE. Photoresponsive crown ethers. 13. Synthesis of photoresponsive NS20 crown ethers and application of the copper (I) complexes to oxygen binding. *Bull. Chem. Soc. Jpn.* 57: 2879–2884, 1984.
172. SIEGELBAUM, S. A., J. S. CAMARDO, AND E. R. KANDEL. Serotonin and cyclic AMP close single K^+ channels in *Aplysia* sensory neurones. *Nature Lond.* 299: 413–417, 1982.
173. SINE, S. M., AND J. H. STEINBACH. Activation of acetylcholine receptors by low concentrations of agonist (abstr.). *Biophys. J.* 45: 386, 1985.
174. SINGH, B. N. Pharmacological basis for the therapeutic applications of slow-channel blocking drugs. *Angiology* 33: 492–515, 1982.
175. SPEDDING, M., AND I. CAVERO. "Calcium antagonists": a class of drugs with a bright future. II. Determination of basic pharmacological properties. *Life Sci.* 35: 575–587, 1984.
176. SPRAY, D. C., A. L. HARRIS, AND M. V. L. BENNETT. Gap junctional conductance is a simple and sensitive function of intracellular pH. *Science Wash. DC* 211: 712–715, 1981.
177. SPRAY, D. C., J. M. NERBONNE, A. CAMPOS DE CARVALHO, A. L. HARRIS, AND M. V. L. BENNETT. Substituted benzyl acetates: a new class of compounds that

reduce gap junctional conductance by cytoplasmic acidification. *J. Cell. Biol.* 99: 174–179, 1984.

178. SPRAY, D. C., J. NERBONNE, J. A. CONNOR, A. L. HARRIS, A. CARVALHO, AND M. V. L. BENNETT. Nitrobenzyl esters reversibly decrease gap junctional conductance (abstr.). *J. Cell Biol.* 95: 109, 1982.
179. STRONG, J. A. Modulation of potassium current kinetics in bag cell neurons of *Aplysia* by an activator of adenylate cyclase. *J. Neurosci.* 4: 2772–2783, 1984.
180. TAKAI, Y., A. KISHIMOTO, Y. IWASA, Y. KAWAHARA, T. MORI, AND Y. NISHIZUKA. Calcium-dependent activation of a multifunctional protein kinase by membrane phospholipids. *J. Biol. Chem.* 254: 3692–3695, 1979.
181. TERANISHI, T., K. NEGISHI, AND S. KATO. Dopamine modulates S-potential amplitude and dye-coupling between external horizontal cells in carp retina. *Nature Lond.* 301: 243–246, 1983.
182. THOMAS, R. C. The role of bicarbonate, chloride and sodium ions in the regulation of intracellular pH in snail neurones. *J. Physiol. Lond.* 273: 317–338, 1977.
183. THOMAS, R. C., AND R. W. MEECH. Hydrogen ion currents and intracellular pH in depolarised voltage-clamped snail neurones. *Nature Lond.* 299: 826–828, 1982.
184. TRIVEDI, B., AND W. H. DANFORTH. Effect of pH on the kinetics of frog muscle phosphofructokinase. *J. Biol. Chem.* 241: 4110–4112, 1966.
185. TSIEN, R. Y. New tetracarboxylate chelators for fluorescence measurement and photochemical manipulation of cytosolic-free calcium concentrations. In: *Optical Methods in Cell Physiology*, edited by P. DeWeer and B. Salzberg. New York: Wiley, 1986, vol. 40, p. 327–346. (Soc. Gen. Physiol. Ser.)
186. TSIEN, R. W., W. GILES, AND P. GREENGARD. Cyclic AMP mediates the effects of adrenaline on cardiac Purkinje fibres. *Nature New Biol.* 240: 181–183, 1972.
187. TSIEN, R. Y., AND R. S. ZUCKER. Control of cytoplasmic calcium with photoisomerizable tetracarboxylate 2-nitrobenzhydrol chelators. *Biophys J.* 50: 843–853, 1986.
188. TURIN, L., AND A. E. WARNER. Carbon dioxide reversibly abolishes ionic communication between cells of early amphibian embryo. *Nature Lond.* 270: 56–57, 1977.
189. UEHARA, A., AND J. R. HUME. Interactions of organic calcium channel antagonists with calcium channels in single frog atrial cells. *J. Gen. Physiol.* 85: 621–647, 1985.
190. VASSORT, G., O. ROUGIER, D. GARNIER, M. P. SAUVIAT, E. CORABOEUF, AND Y. M. GARGOUIL. Effects of adrenalin on membrane inward currents during the cardiac action potential. *Pfluegers Arch.* 309: 70–81, 1969.
191. WALDMAN, S. A., R. M. RAPOPORT, AND F. MURAD. Atrial natriuretic factor selectively activates particulate guanylate cyclase and elevates cyclic GMP in rat tissues. *J. Biol. Chem.* 259: 14332–14334, 1984.

191a. WALKER, J. W., J. A. MCCRAY, AND G. P. HESS. Photolabile protecting groups for an acetylcholine receptor ligand. Synthesis and photochemistry of a new class of *o*-nitrobenzyl derivatives and their effects on receptor function. *Biochemistry* 25: 1799–1805, 1986.

192. WANKE, E., E. CARBONE, AND P. L. TESTA. K^+ conductance modified by a titrable group accessible to protons from the intracellular side of the squid axon membrane. *Biophys. J.* 26: 319–324, 1979.
193. WASSERMANN, N. H., E. BARTELS, AND B. F. ERLANGER. Conformational properties of the acetylcholine receptor as revealed by studies with constrained depolarizing ligands. *Proc. Natl. Acad. Sci. USA* 76: 256–259, 1979.
194. WASSERMANN, N. H., AND B. F. ERLANGER. Agents related to a potent activator of the acetylcholine receptor of *Electrophorus electricus*. *Chem. Biol. Interact.* 36: 251–258, 1981.

194a. WEINSTOCK, M. M. Activation and desensitization of acetylcholine receptors in fish muscle with a photoisomerizable agonist. *J. Physiol. Lond.* 338: 423–433, 1983.

195. WRIGHT, E. M. Mechanisms of ion transport across the choroid plexus. *J. Physiol. Lond.* 226: 545–571, 1972.
196. WRIGHT, E. M. Effect of bicarbonate and other buffers on choroid plexus Na^+/K^+ pump. *Biochim. Biophys. Acta* 468: 486–489, 1977.
197. YAMAMURA, H. I., H. SCHOEMAKER, R. J. BOLES, AND W. R. ROESKE. Diltiazem enhancement of [^{3}H]nitrendipine binding to calcium channel associated drug receptor sites in rat brain synaptosomes. *Biochem. Biophys. Res. Commun.* 108: 640–646, 1982.
198. YELLEN, G. Single Ca^{2+} activated nonselective cation channels in neuroblastoma. *Nature Lond.* 296: 357–359, 1982.
199. ZELIS, R., AND S. F. FLAIM. Calcium blocking drugs for angina pectoris. *Annu. Rev. Med.* 33: 465–478, 1982.
200. ZUCKER, R. S., AND R. Y. TSIEN. A new method for changing intracellular calcium concentration. *Soc. Neurosci. Abstr.* 11: 791, 1985.