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Instruments and techniques

A low cost high intensity flash device for photolysis experiments

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Abstract. Novel techniques of flash photolysis experiments require high intensity light sources in the near UV. We describe here a simple and inexpensive flash device which may compete with bulky and expensive laser systems if the experiments do not necessitate very short light pulses. Using a particular optical arrangement and stored electrical energy, a variation of the parameters voltage and capacitance led to a difference in light output by a factor of more than two. The system is used to relax both skeletal and smooth muscle fibres in the rigor state by releasing up to 2 mM ATP from 12.5 mM caged-ATP.

Key words: Muscle – ATP-jump – Caged-ATP – Flash photolysis – Caged nucleotides

Introduction

The availability of photosensitive reactants leads to a variety of novel experiments in the life sciences. A number of them may be used in physiological and pharmacological experiments and are reviewed by Lester and Nerbonne (1982). One of the first applications were concentration jumps of ATP induced by photolysis of the so-called caged-ATP, a photolabile precursor (Kaplan et al. 1978). These experiments opened a number of new approaches to the investigation of the mechanism of muscle contraction (McCray et al. 1980; Goldman et al. 1984; Rapp et al. 1986).

Common to all the photolysis experiments in the muscle field, a high intensity light flash in the near UV is needed to release the nucleotides within a short period of time, thus producing a concentration jump of these nucleotides. Powerful laser systems producing UV light of the appropriate wavelength range can serve as ideal sources with high energy density and ideal pulse structure for flash experiments. Their main disadvantages are their difficulty of maintenance, their weight and size and last their high cost, both of purchasing and maintenance.

Commercially available Xe-gas discharge systems on the other hand suffer from lack of sufficient UV intensity. The aim of this article is to describe a system consisting of a power supply, flashlamp and UV optics suitable for flash photolysis and which can be made at reasonable cost. The system is simple, lightweight and transportable. It is currently used for relaxation and structural experiments in muscle research.

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Materials and results

Selection of bulb-type

Standard flashlamps range from linear and helical shaped bulbs to so-called point sources, where the electrodes are just a few millimeters apart. There are a variety of suppliers of the items. Considerations related to light output, which depends mainly on gas fill and pressure of the bulb and on the electronic circuit as well as optical aspects, led the authors to select a high pressure Xe point source lamp. We compared various bulb types with electrode separations from 1.5 mm to 3.0 mm and fill pressures from 0.5 bar to more than 3 bar from different suppliers with respect to their conversion efficiency of electrical energy to UV light. The most efficient standard lamp tested was lamp model 35 S from Chadwick Helmuth, El Monte, CA, USA. The inner bore diameter is 6 mm, the outer 10 mm, the electrode separation is 3 mm. Although the fill pressure is proprietary, we estimate from the spectral data a pressure of 2–3 bar or more.

When the bulbs are operated in flash mode, inert gases show certain advantages over mercury and metal halide lamps. Due to its low ionisation energy compared with other inert gases, Xe converts electrical energy into light most efficiently (for references concerning gas discharges see Phillips 1983). The spectral output is mainly dependent on the current density and fill pressure, small values of both (0.26 atm Xe, 2870 A/cm^2) leading essentially to a line spectrum with dominating lines in the near infrared (800–1,000 nm). In order to shift the spectral output of a given gas discharge towards the UV part of the spectrum, either high current densities or high fill pressures or a combination of both are suitable (Goncz and Newell 1966).

Electrical parameters

The basic circuit diagram for the flashlamp is shown in Fig. 1. A bank of low inductance capacitors suitable for rapid discharge is charged to a given voltage, which determines together with the inductance and resistance of the circuitry the pulse duration and the current density at the instant of the discharge. As long as the capacitor voltage is lower than the self-ignition voltage, the lamp acts as an infinitely large resistor. Ignition is triggered by a high voltage pulse either by an external wire wrapped around the bulb or by a pulse in series with the main discharge pathway. External triggering devices are simple in construction, but series triggering is more reliable and gives better pulse to pulse reproducability with respect to both conversion of electrical

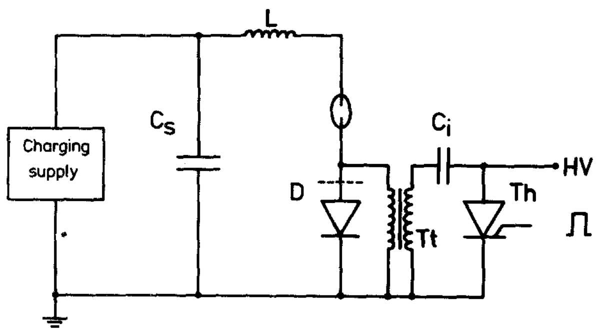

Fig. 1. Principal diagram of the electrical circuit with storage capacitor C_s, current limiting inductance L, holdoff diode D, triggertransformer Tt, ignition capacitor C_i and thyristor Th. Designing a high voltage setup with the series trigger transformer separated from the main discharge pathway with a set of holdoff diodes, a small inductance of 5–10 μH is necessary to limit current rise. With this design the pulses are shorter than 200 μs. The inductance is not necessary in the low voltage configuration using this trigger design, but the pulse length exceeds 2.5 ms. In a further design, the trigger transformer is part of the main pathway (no hold off diodes required). Our final setup has the following specifications: C_s = 3,290 μF, charged to 350 V which gives a total of 200 J of stored electrical energy. No holdoff diode, trigger transformer with a ratio of N_p:N_s = 1:45. C_i = 0.3 μF, charged to 600 V, AC type

energy into light and of the geometry of the discharge arc between the electrodes.

As indicated in Fig. 1, series triggering is possible with the trigger transformer in the main pathway or separated by a set of holdoff diodes. These have to block the ignition voltage (10–15 kV) and carry all the current during discharge. Using a transformer as part of the main pathway results in a higher inductance of the circuit and therefore a longer pulse and a lower current density.

We compared several setups varying voltage, capacitance and inductance using series triggering with respect to their spectral and temporal behaviour.

Measurements of the light intensity at different wavelengths indicate that the number of photons is increased by reducing the voltage and increasing the capacity, irrespective of which part of the spectrum is considered and independent of the trigger type used. For two setups (U = 298 V, C = 4,500 μF, L < 1 μH and U = 894 V, C = 500 μF, L = 5 μH resp.), data were sampled with a monochromator (bandwidth 5 nm) and a silicon photodiode with amplifier. Although the diode measures photon flux, the important parameter for flash photolysis experiments is the total energy irradiated. Therefore the peak value of the photocurrent was multiplied by the duration of full width at half maximum [approx. 130 μs for the high voltage design (U = 894 V) and 750 μs for the low voltage case (U = 298 V) resp.]. The spectra are shown in Fig. 2. The irradiated energy obtained at a low voltage is at all wavelengths considerably higher than that at a high voltage. The maximum of emission is slightly shifted towards the blue from 450 nm to 425 nm in the high voltage design. With a condensor used as described in Fig. 4 and the bolometer placed close to the condensor, we measured an integral intensity of 1.25 J per pulse in the low voltage setup. Relating this value to the spectral distribution (Fig. 2), we yield approx. 2.7 mJ at wavelengths λ < 300 nm (0.2%), 210 mJ in the range from 300 to 400 nm (16.5%), 880 mJ in the visible range from 400 nm to 700 nm (70%) and about 160 mJ in the infrared part of the spectrum above 700 nm (13.3%). The wavelengths most efficient for

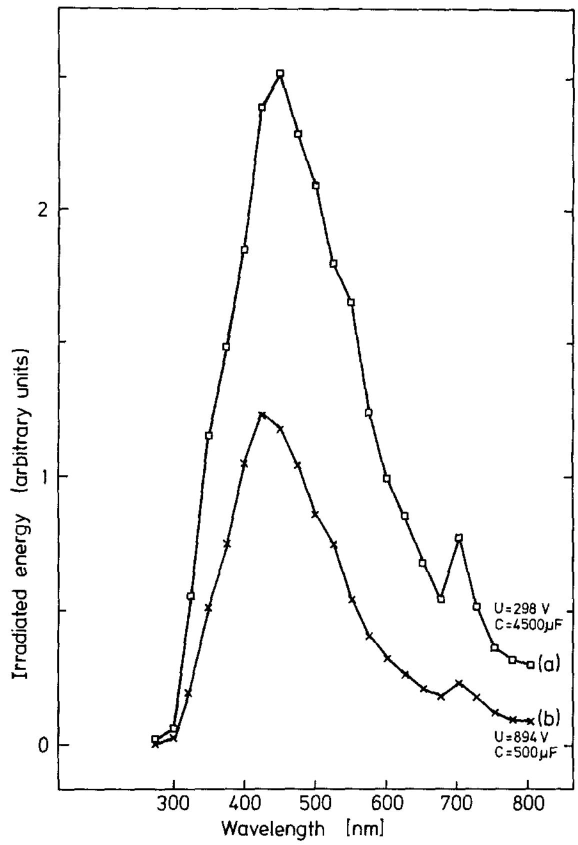

Fig. 2. Spectra of low (a, 298 V, 4,500 μF, L < 1 μH) and high voltage (b, 894 V, 500 μF, L = 5 μH) design. Data from the photodiode were corrected for photodiode sensitivity using the calibration curve provided by the manufacturer (Hamamatsu, Japan) and multiplied with the time at full width at half maximum to get energy converted data. Each value was measured twice

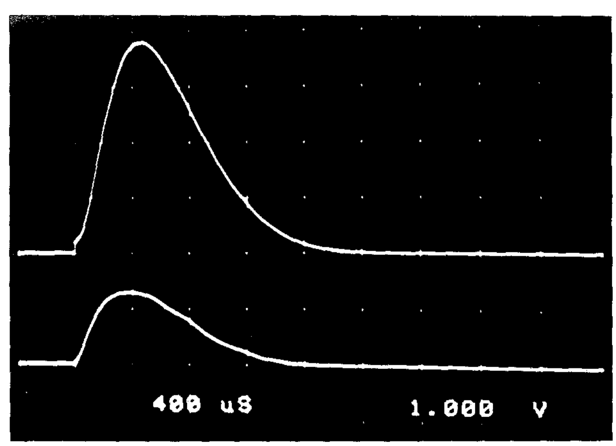

Fig. 3. Time structure of discharge (*upper trace*) and of light pulse (*lower trace*). Time scale is 400 μs per division, sensitivity is 1 V per division. To measure current density, a resistor of 0.071 Ω was placed in series of the discharge on the grounded part of the circuit. The *upper trace* shows the voltage drop across this resistor using a 1:10 probe. Peak voltage is 38 V, yielding a current of 535 A at peak

photolysis experiments (300 nm–400 nm) were selected from the spectrum by a UG 11 filter. A 3 mm thick filter of this type is highly transmittant for UV light (λ < 400 nm) and has also a low transmittance in a band centered at 700 nm (T < 3% at 700 nm).

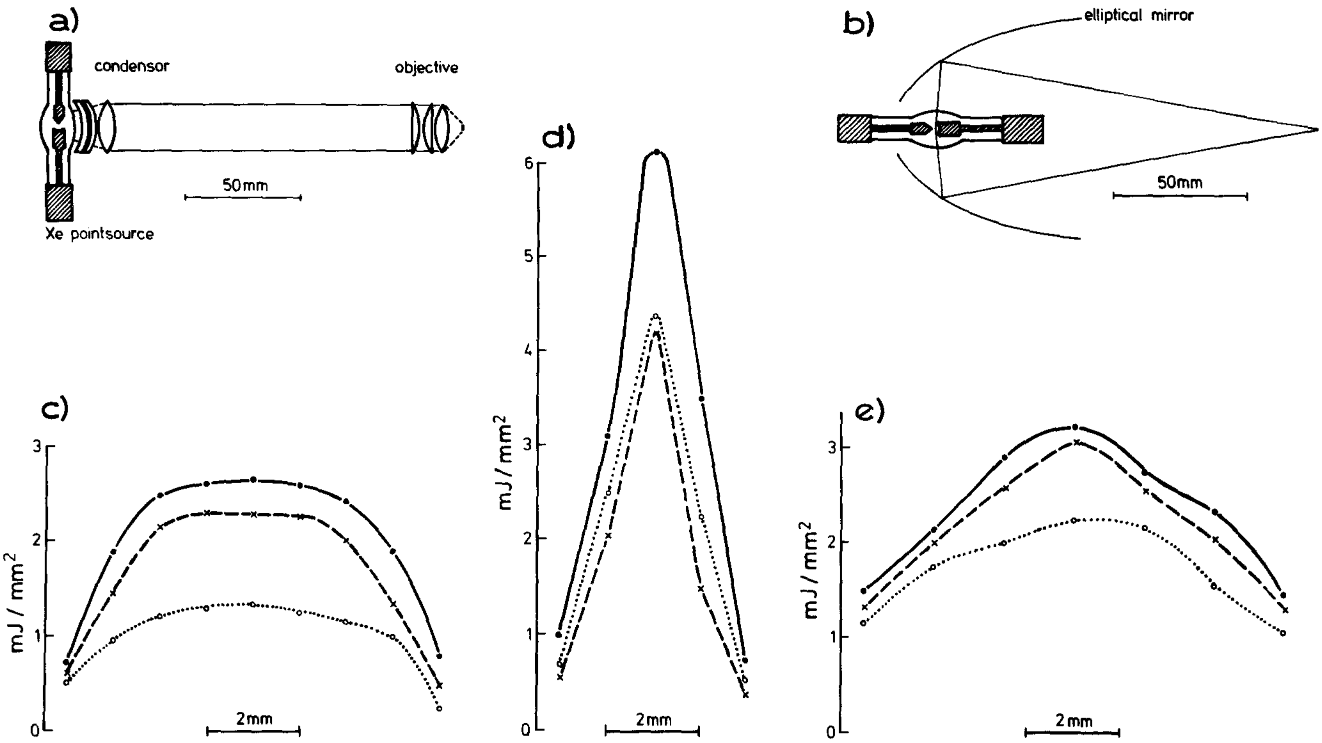

Fig. 4. Optical arrangement (**a**, **b**) and energy densities in the focus in the wavelength range selected with a 3 mm thick UG 11 filter (**c**, **d**, **e**). The focii were scanned with a 1×1 mm^2 aperture in horizontal and vertical steps of 1 mm (**c**, **d**) and in 1.5 mm steps (**e**). *Straight lines* indicate energy density in the focal plane, *broken lines* are the values 1.0 and 2.0 mm above (**c**), 1.0 mm below and 1.0 mm above (**d**) and 1.5 and 3.0 mm (**e**) above this plane. In the setup using lenses, a condensor, consisting of 2 concave-convex lenses (f = 50 mm) and one beconvex lens (f = 25 mm) covers a solid angle of about 1.8 steradian (equal to approx. 15% of a sphere). Using an objective of 2 plane convex lenses (f = 40 mm) and a biconvex lens (f = 25 mm) produces an image 5 mm distant from the last lens. Data for this setup are shown in (**d**). This design gives a high energy density and a small but sharply pointed focus. For the muscle experiments, an objective, consisting of 1 plane convex lens (f = 40 mm) and a cylindrical lens (f = 65 mm) was used. The distance between the last lens and the focus is 25 mm, the energy density is much lower (**c**), but in the horizontal direction homogeneous over 6 mm. All lenses are of diameter 22.4 mm. Since only about 15% of the irradiated light could be focussed, a setup using an elliptical mirror (**b**) was compared with the other optics. The focus size is much bigger, the energy density in the focus is in between the value of the other setups

The advantage of higher energy output in the low voltage configuration (see Fig. 2) is paralleled with the disadvantage of longer pulse durations due to the discharge behaviour of capacities in a R-C combination (approximately 2.5 ms at 1/10 maximum intensity compared with 200 µs in the high voltage case). This might be a significant limit in time resolution of the corresponding experiments. The situation is improved by using a trigger transformer as part of the main pathway (R-C-L combination) and a slightly higher voltage. In Fig. 3 the current density of the discharge is measured by using a series resistor (R = 0.71 Ω) and simultaneously the light pulse is recorded by means of a photodiode. The upper trace shows the voltage across the resistor. Since the diameter of the electrodes is 4 mm and assuming the same value for the discharge, we get approx. 4,250 A/cm^2. This is consistent with the results of Gonzc and Newell. The pulse duration for both the current and the light output is approx. 1.2 ms at 1/10 of the maximum.

Optical considerations

The choice of a point source lamp was mainly for optical reasons. Standard linear flashlamps which could be operated with the same power as these bulbs have much larger dimensions, which causes difficulties in focussing onto the sample or require at least expensive high aperture quartz lenses.

In order to achive a high energy density on the sample, we tested one setup using quartz optical elements and another using an elliptical mirror with the bulb in one focal point and the sample in the other. Figure 4 summarizes the results. Since we use the setup for experiments on up to 5–6 mm long muscle fibres, a cylindrical lens was used to achive an elongated elliptical focus. The energy density was measured with an aperture of 1×1 mm^2 and a UG 11 filter. This setup shows the lowest energy density (Fig. 4c) but a homogeneous focus over the full length of the muscle fibre. The focus is also in a convenient distance of 25 mm from the last lens. One can improve the energy density of more than a factor of two, if small point shaped samples are used (Fig. 4d). With an elliptical reflector as a focussing element, about 80% of the irradiated energy is collected (compared to approx. 15% with the condensor used), but it was not possible to achieve a comparable small focus size, therefore resulting in no appreciably higher energy density (Fig. 4e). Which type of optics is appropriate is mainly dependent on sample size and geometrical limitations of the setup.

Application

For flash photolysis experiments with a temporal resolution of 1 ms or less and for the type of bulb discussed here, the use of a low driving voltage and a high capacity is

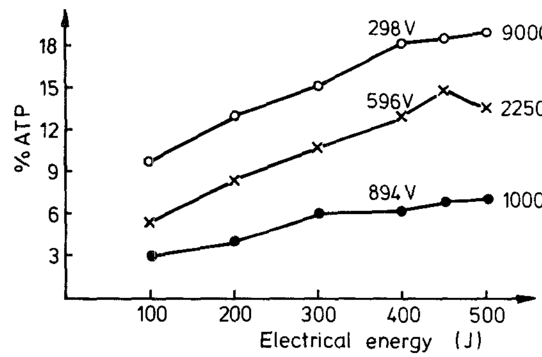

Fig. 5. Dependence of degree of ATP photolysis from 12.5 mM caged-ATP for different circuit designs in a configuration with a series trigger transformer separated by a set of hold-off diodes. Each *point* is an average of two experiments. Caged-ATP was photolysed in a $1 \times 1 \times 8\ mm^3$ cuvette using an optical setup similar to the described one in Fig. 4a with cylindrical lens. Concentration gradients of ATP in this cell due to the high concentration of caged-ATP and the long pathway were averaged in the analysis of photolysis where 2 µl of solution were taken. This means that the amount of ATP released in the first layers of the solution is even higher. ATP production from caged-ATP was assayed by HPLC on a Polyol Si 60 RP-18 column (4.25 × 62.5 mm, Serva, Heidelberg, FRG) using an acetonitrile gradient in 50 mM phosphate buffer (pH 6.0)

recommended. The described setup is currently used to relax smooth muscle (Arner et al. 1986) and skeletal muscle (Rapp et al. 1986) from the rigor state by releasing up to 2 mM ATP from 12.5 mM caged-ATP. The latter experiments were performed in conjunction with structural investigations by monitoring the time-course of the strongest equatorial and meridional reflections in the X-ray pattern after ATP-jump. Figure 5 shows the amount of ATP released from a 12.5 mM caged-ATP solution as a function of stored electrical energy at different capacities. It confirms very clearly the spectral data. Doubling the stored electrical energy by doubling the capacitance is possible but increases the energy density only about 50 to 70%, since circuit losses become more dominant and since the discharge then fills the whole volume of the bulb which results in a bigger focus. Using higher stored electrical energy reduces the lifetime of the bulb largely.

In more recent experiments we applied this setup for the photogeneration of Ca^{2+} and ATP-γ-S. Further photolysis studies of other authors include photogeneration of ADP (Y. E. Goldman: pers. communication) and of phosphate (Dantzig et al. 1987).

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