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A system for applying rapid warming or cooling stimuli to cells during patch clamp recording or ion imaging

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Abstract

We describe a system for superfusing small groups of cells at a precisely controlled and rapidly adjustable local temperature. Before being applied to the cell or cells under study, solutions are heated or cooled in a chamber of small volume (~ 150 μl) and large surface area, sandwiched between four small Peltier elements. The current through the Peltier elements is controlled by a microprocessor using a PID (proportional-integral-derivative) feedback algorithm. The chamber can be heated to at least 60 °C and cooled to 0 °C , changing its temperature at a maximum rate of about 7 °C per second; temperature ramps can be followed under feedback control at up to 4 °C per second. Temperature commands can be applied from the digital-to-analogue converter of any laboratory interface or generated digitally by the microprocessor. The peak-to-peak noise contributed by the system does not exceed that contributed by a patch pipette, holder and headstage, making it suitable for single channel as well as whole cell recordings.

Keywords: Temperature control; Patch clamp recording; Fluorimetry; Peltier device; Feedback control

1. Introduction

When recording electrical signals or ion concentrations in individual cells in vitro, stable control over temperature is usually required. If the temperature is to remain the same for the whole experiment or to change only slowly, this aim can be achieved using a number of commercially available bath temperature controllers. Changing the temperature rapidly then holding it accurately at a desired level is more difficult, especially if cells are to be cooled as well as heated. Systems for rapid heating of superfusing solutions have been described (Datyner et al., 1985; Dittert et al., 1998); in the latter system, solutions pass through a small-diameter tube coated with a platinum heating element, which can change solution temperature at the remarkable rate of up to 0.1 °C/ms (100 °C/s) with a settling time of less than 1 s after a 30 °C step change in temperature. Systems for accurate bipolar temperature control (i.e. both cooling and heating) have mostly been based on Peltier elements, used either directly around the bath and/or inlet tubing (Corrèges et al., 1998; Forsythe and Coates, 1988) or indirectly, via a refrigerant (Datyner and Cohen, 1991). The Peltier element is a heat exchanger; when current is passed through it, one face becomes warmer and the other cooler, and when the direction of current flow is reversed, the direction of heat exchange is also reversed. Feedback control of temperature is achieved by controlling the current through the Peltier elements.

We describe here a Peltier-based system which was designed for a study of thermal transduction in isolated sensory neurones. For this purpose, it was necessary to apply accurate thermal stimuli with rates of change substantially faster than the known rates of adaptation of skin thermoreceptors (Kenshalo, 1970). Since the work involves both patch clamp recording (at the whole cell and single channel level) and imaging of intracellular ion concentrations in groups of cells, the system also had to satisfy requirements of low electrical noise and the ability to sustain a relatively high flow rate, sufficient to keep an area containing 5–10 cells at a uniform

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temperature. No commercially available system satisfied all our requirements, making it necessary to design the system presented here.

2. Methods

2.1. Construction of the device: heater/cooler assembly and temperature measurement

The assembly for heating or cooling solutions is shown in Fig. 1. Test solutions pass through a small flat chamber sandwiched between Peltier elements, where they are heated or cooled. The exit of the heating/cooling chamber is placed as close as possible to the cell or cells under study, to minimise heat exchange with the surroundings;

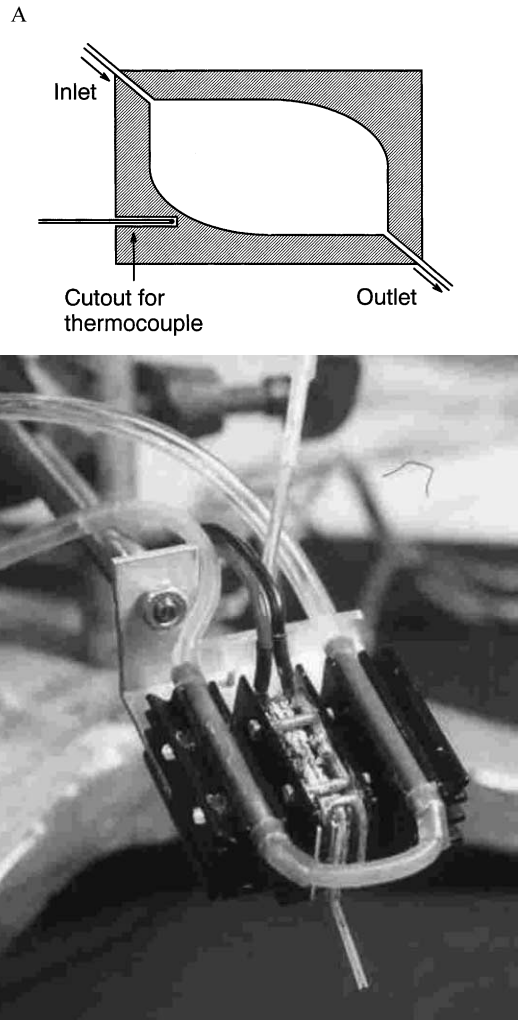

Fig. 1. The application system and chamber. (A) The shape of the heating/cooling chamber (dimensions 15 mm × 30 mm × ~1 mm thick), showing the cutout for the thermocouple K_2. The polystyrene spacer (1 mm thick) which separates the coverslips is shaded; the unshaded part is the hollow space between the two coverslips which is filled by the solution. (B) The device, assembled. The heating/cooling chamber is sandwiched between the Peltier devices, which are visible between the heatsinks. Solution enters the heating/cooling chamber through the tubing at the top and leaves at the bottom; the outlet tubing at the bottom is placed ~1 mm from the cell or cells under study. The whole device measures 32 mm long × 40 mm wide × 23 mm high.

after leaving the chamber, the solution travels only ~2 cm to the cell.

The shape of the flat heating/cooling chamber is shown in Fig. 1A. Its volume is small (~150 μl) and its surface area is large, to maximise the efficiency of heat transfer. It is constructed from two borosilicate glass coverslips (no. $1\frac{1}{2}$, 0.16–0.19 mm thick; Chance-Propper Ltd., Smethwick, UK) separated by a polystyrene spacer about 1 mm thick cut from the lid of a 35 mm culture dish (Corning, cat. no. 29000, obtained from Sigma), and glued together with standard epoxy resin adhesive.

The heating/cooling chamber is sandwiched between four Peltier devices, two on each side, wired in series (see Fig. 1B); capacitive coupling between the Peltier current and the solution is avoided by introducing thin grounded aluminium foil between the Peltier devices and the chamber. Each Peltier device (RS Components, Corby, UK; cat. no. 197-0332) measures 15 × 15 mm and is rated at 9.2 W. The Peltier devices are mounted between aluminium heatsinks cut from standard TO-220 heatsinks (RS Components, cat. no. 402-995); all mating surfaces are coated with heatsink compound. The heating and cooling unit is compact (measuring 32 mm long × 40 mm wide × 23 mm high) and designed to be placed next to the recording bath, below the condenser of the inverted microscope used for recording and imaging (we use the Nikon Eclipse TE200 and Olympus IX70). The height of the unit was kept to a minimum in order to fit within the working distance of the condenser (about 50 mm). The outlet tubing (1 mm inside diameter; bottom of Fig. 1B) is about 2 cm long and its exit is placed about 1 mm from the cell. The heater/cooler assembly is mounted in a coarse manipulator (Märzhäuser, Wetzlar, Germany; model MM33) to allow easy positioning near the cell. We normally use a flow rate of 1–2 ml/min, and apply solutions locally to a single cell or a group of cells in a bath of volume ~1 ml. The system could also be used to control the temperature over the whole of a smaller bath.

Before being heated or cooled in the chamber, the solution is passed through a manifold constructed from Teflon intravenous cannulae (20 gauge; Vygon GmbH, Aachen, Germany), allowing up to 12 solutions to be switched using stopcocks or miniature solenoid valves. The resistance of the manifold is low, and after the manifold a resistance element is added, cut from a Microloader (Eppendorf GmbH, Hamburg, Germany, cat. no. 5242 956.003) to the appropriate length for the desired flow rate.

A type K thermocouple made from 0.2 mm wire (RS Components, cat. no. 219-4315) is placed in a small cutout in the polystyrene spacer (see Fig. 1A), within 1 mm of the edge of the heating/cooling chamber, in contact with the inside surface of the coverslips. It is the temperature at this point (labelled K_2 in Fig. 2) that is normally controlled by the feedback controller, because this allows the most rapid feedback without

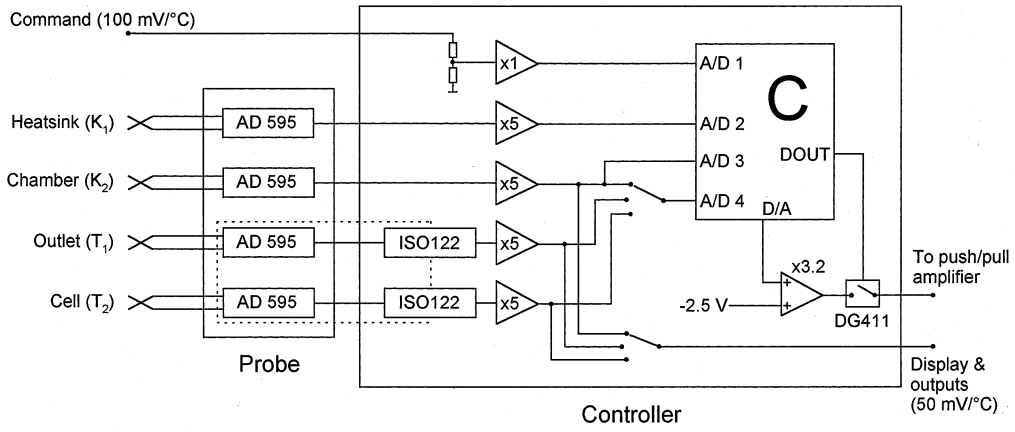

Fig. 2. Block diagram of the temperature controller. Integrated circuits are used as described in the manufacturers' data sheets. The probe is mounted inside the Faraday cage of the recording setup and connected to the controller by a screened cable. Power for the isolated part of the circuit (enclosed by the dotted line) is from two 9 V rechargeable batteries, regulated to ± 7.2 V; other power supplies are conventional ± 12 V and $+5$ V regulated supplies. Temperature is measured at four thermocouples, two type K (K_1 and K_2) which are always used, and two type T (T_1 and T_2) which are optional (see text). The C-control microprocessor (labelled 'C') compares the analogue command signal at analogue/digital (A/D) input 1 with the actual temperature either at the chamber (measured with thermocouple K_2), in the outlet tubing (thermocouple T_1) or in the bath next to the cell (thermocouple T_2); this signal is fed into A/D input 4. The difference between actual and command temperatures, and the integral and derivative of this difference, are used to generate the input to the push-pull amplifier [see Eq. (1)]; since the output of the microprocessor is a 0–5 V signal, this is fed to a summing amplifier (gain $\times$ 3.2) to convert it into the $-8-+8$ V signal required by the push-pull amplifier. AD595 are integrated thermocouple amplifiers and ISO122 are isolation amplifiers. The $\times 5$ amplifiers are $\frac{1}{2}$LM353 for K_1 and K_2, and LM356 for T_1 and T_2; the gain of the latter amplifiers is adjustable and the offset trimming of the LM356 is used, allowing the inaccuracies in the AD595s to be corrected and the temperature to be measured with an error of less than ± 0.5 °C over the range 10–50 °C. The actual temperature measured by K_2, T_1 or T_2 can be continuously displayed on a digital voltmeter (DVM) and fed to the laboratory interface as an analogue signal (50 mV/°C); several switchable outputs are used (only one shown), allowing more than one temperature to be simultaneously recorded, and the temperature displayed on the DVM to be switched between K_2, T_1 and T_2 independently of the analogue outputs. The temperatures at the heatsink and chamber are continuously monitored (at A/D inputs 2 and 3) and, if either of them exceeds a preset level, the digital output DOUT opens the DG411 analogue switch and disconnects the controller from the push-pull amplifier. Other components not shown are (a) LED indicators (driven from other digital outputs of the C-control unit) to show the status of the unit (cooling, heating or disconnected because the set temperature was exceeded); (b) a second DVM output to display the command or heatsink temperature; and (c) the digital inputs on the C-control unit which are used to trigger temperature stimuli independently of the analogue input (see text).

introducing excess noise; the temperature here is, however, not the same as that at the cell (see Fig. 4A and Fig. 6). A second identical thermocouple (K_1) is mounted in a hole drilled into one of the heatsinks; to protect the device, if the temperature at either K_1 or K_2 exceeds a preset value, the controller is shut down. In addition, if desired, the solution temperature can be measured at the outlet of the device or beside the cell (or both) using miniature type T thermocouples; either of these thermocouples (T_1 and T_2 in Fig. 2) can also be used as the reference point for temperature control, allowing the cell temperature to be controlled more accurately at the cost of slower feedback and increased electrical noise. The type T thermocouples are commercially available (Physitemp, Clifton, NJ, USA, cat. no. IT-1E) or else laboratory made from 50 μm thermocouple wire (Physitemp). In the latter case, the wires are welded under a binocular zoom microscope by discharging a 1000 μF capacitor charged to 24 V onto the tips of both wires simultaneously, from a pointed tungsten wire used as welding electrode. In practice we do not normally use the type T thermocouples during patch clamp experiments; we measure only the chamber temperature and control the temperature from this point, and estimate the cell temperature afterwards by repeating the thermal stimuli with T_2 placed where the cell had been. This avoids introducing noise during recording or releasing ions from the thermocouple wire that could affect the physiological process under study.

2.2. Construction of the device: the temperature controller

A block diagram of the controller is shown in Fig. 2. All thermocouples are connected to thermocouple amplifiers (Analog Devices AD595AQ), and the type T thermocouples, which are in direct contact with the recording solution, are isolated from the rest of the device and from the recording ground using isolation amplifiers (Burr-Brown ISO122P; all integrated circuits were obtained from RS Components). The actual temperature (measured at K_2, T_1 or T_2) is compared with the desired temperature, and the controller adjusts the current through the Peltier devices in order to keep them the same. The temperature command is normally an analogue voltage (100 mV/°C) which can be gener-

ated by a potentiometer or, more conveniently, by the digital-to-analogue output of any laboratory interface. If two analogue outputs are available on the laboratory interface, voltage or current commands can be applied to a patch clamp amplifier from one channel, and temperature commands can be simultaneously applied to the temperature controller from the second channel (see Fig. 4B). The software used in the recordings presented here was written by GR for the Labmaster DMA interface (Scientific Solutions, Mentor, OH, USA).

The controller is based on a Motorola microprocessor which can be programmed very conveniently in a dialect of BASIC (C-Control system; Conrad Electronic, Hirschau, Germany). The program uses the PID (proportional-integral-derivative) method of control: the command signal is generated from the difference between the actual and desired values, along with the integral of this difference (which makes the control more accurate over long periods of time) and its derivative (which allows the controller to deal with rapid changes and also counteracts oscillations). These three variables are weighted with the appropriate coefficients and used to generate a value for the voltage to be fed to the Peltier devices (-8 V to $+8$ V). The PID equation is used in the program in its simplest form:

$$CO_t = Pe_{t-1} + I\left(\int e_{t-1}dt\right) + D(d/dt\, e_{t-1}) \quad (1)$$

where:

CO_t is the controller output in volts at a given time;

e_{t-1} is the error (command − actual temperature) at the previous time step (~ 20 ms); and

P, I and D are weighting coefficients for the proportional, integral and derivative terms.

This command voltage is filtered and then drives a conventional transistor push-pull amplifier to supply the necessary current to the Peltier devices. The push-pull amplifier is constructed using 2N3773 (NPN) and MJ2955 (PNP) power transistors whose base current is supplied by a TDA2030 operational amplifier (RS Components); we found it unnecessary to make any provision for reducing crossover distortion. The response of the power transistors is slowed using 10 μF tantalum capacitors between collector and base.

The microprocessor can also be programmed to give command signals other than that derived from the analogue command, in response to a digital trigger. This feature can be used to generate virtually any desired waveform of thermal stimulus (limited only by the 8 kilobyte memory of the microprocessor), and therefore allows the system to be used independently of a laboratory interface if none is available, or if the laboratory interface has no free analogue output.

Full circuit diagrams and the PID program are available on request from GR.

3. Performance of the system

Responses to step changes in temperature (heating and cooling) are shown in Fig. 3A. There is an initial fast phase (up to 7 °C per second) and, as the command temperature is approached, the rate of temperature change slows so that the overshoot is minimal. The temperature settles to its final value within 5–15 s (depending on the size of the temperature jump). Temperature measured at the exit tubing follows that at the chamber with a slight lag due to heat transfer through the coverslips and the transit time of the solution, and never reaches the chamber temperature because of heat exchange with the bath solution and air (Fig. 3A, lower, and Fig. 6). For studies on thermal transduction, a ramp command is often the most useful stimulus. Ramps can be followed under feedback control, at rates from below 0.5 °C/s (Fig. 3B) up to a maximum rate of 4 °C/s. The rates of temperature change achieved at the cell with this system are about 5–10 times faster than described for systems where the whole bath was heated or cooled (Corrèges et al., 1998; Kobayashi and Takahashi, 1993).

Fig. 4 shows whole cell recordings obtained with the system in studies of cold thermal transduction (A; see Reid and Flonta, 2001) and ion channel kinetics (B). In Fig. 4A a train of action potentials and the underlying depolarisation induced by cooling in a cultured rat dorsal root ganglion (DRG) neurone is shown. Here the stimulus is simple, a single cooling pulse of 15 s duration. A more complex protocol was used in Fig. 4B

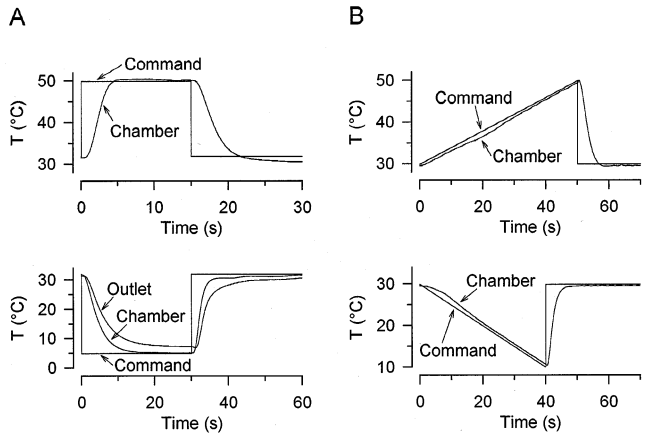

Fig. 3. Performance of the temperature controller. (A) Response to step temperature commands. The analogue command was stepped from a base temperature of 32 °C to 50 °C (above) and 5 °C (below; trace labelled 'Command'). The temperature measured at the heating/cooling chamber (with thermocouple K_2) is shown in both cases (trace labelled 'Chamber'). In addition the lower recording also shows the temperature at the outlet of the system (measured with thermocouple T_1; trace labelled 'Outlet'). Here the solution flow rate was about 2 ml/min. (B) Response to ramp commands at a rate of 0.4 °C/s, from 30 °C to 50 °C (above) and from 30 °C to 10 °C (below). Traces are labelled as in A.

to measure the temperature dependence of slow inactivation of K^+ currents in another DRG neurone. Here two analogue output channels were used on the laboratory interface: one controlled the patch clamp amplifier, giving the holding potential and step voltage pulses, while the second was connected to the temperature control system, and changed the temperature by ~4 °C between each voltage pulse. Allowing 5 s between pulses for the temperature to stabilise and for recovery from inactivation, and using voltage pulses of 5 s duration, the whole recording was acquired in a time of just under 2 min.

The system is also in regular use for imaging of intracellular calcium concentrations ($[Ca^{2+}]_i$) during thermal and pharmacological stimuli. A sample recording is shown in Fig. 5, showing one cold-responsive and one unresponsive neurone during a cooling ramp.

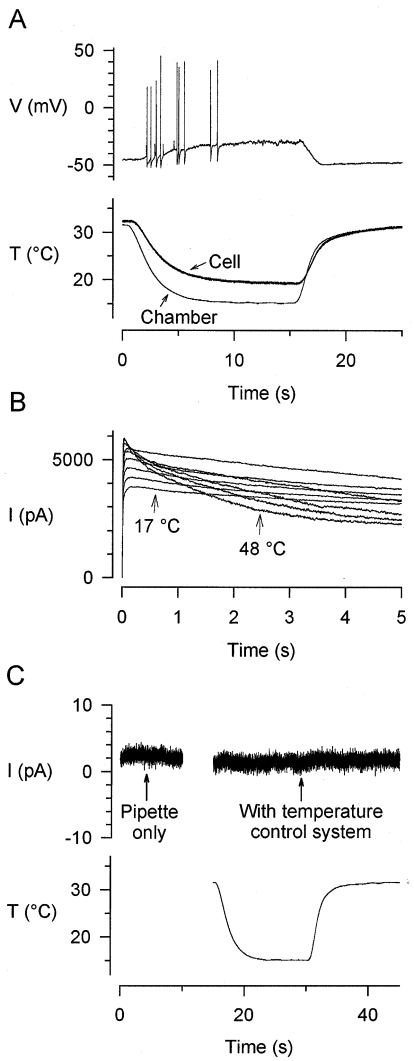

Fig. 4.

4. Additional design considerations

4.1. Electrical interference

A very important requirement of the device is that it should not introduce excess noise into patch clamp recordings. Careful grounding is the most obvious safeguard: the heatsinks, other metal parts of the system and the manipulator in which it is mounted are grounded to the microscope (which in our case is normally connected to the headstage ground connection on the patch clamp amplifier). One terminal of the series-connected Peltier devices is connected to the chassis ground of the amplifier, and the current through the Peltier devices is stabilised by filtering the input of the push-pull amplifier and slowing the response of the power transistors as described above. To remove the possibility of 100 Hz ripple which might occur when drawing a large current from a conventional unregulated mains power supply, the power supply for the push-pull amplifier which provides the Peltier current is obtained from rechargeable Dryfit batteries (12 V, 5.7 Ah; RS Components, cat. no. 591-944). With our standard base temperature of 32 °C and a room temperature of 20 °C, these batteries allow the system to be used continuously for about 12 h before recharging. If longer periods of use are necessary, or if the base temperature is higher (e.g. 37 °C) or well below room temperature, batteries of larger capacity would perhaps be advisable. A pair of stabilised high-current bench

Fig. 4. Electrophysiological recordings made with the temperature control system. (A, upper trace) Depolarisation and action potentials in a cultured rat dorsal root ganglion (DRG) neurone in response to a cooling stimulus (some action potentials are truncated due to the 300 Hz sampling rate); (lower trace) the temperature stimulus. The trace labelled 'Chamber' was measured during the recording at the heating/cooling chamber (with thermocouple K_2; thin line), and that marked 'Cell' after the experiment, by placing a miniature type T thermocouple (T_2) in the position where the cell had been (see text), and repeating the thermal stimulus (thick line). (B) Slow inactivation of K^+ currents in another DRG neurone. Depolarising pulses of 5 s duration to +40 mV were applied from a holding potential of −80 mV, starting at 17 °C. Immediately after each pulse the temperature was increased by ~4 °C, and a 5 s interval was allowed between the pulses for temperature stabilisation and recovery from inactivation. The whole recording, with nine pulses, was acquired in less than 2 min. (C) Baseline noise recorded with a patch pipette in the bath, polished so as to close the tip (simulating a gigaseal). The pipette was fabricated from thin-walled borosilicate glass (GC150TF, Clark Electromedical Instruments, Pangbourne, UK) and not coated; this is the standard type of pipette we use in this laboratory for whole cell recording. The current before introducing the temperature controller into the bath is shown in the first part of the recording; sampling was then paused, the temperature controller lowered into the bath and the solution flow started, and the current recorded during a cooling stimulus (temperature measured at the chamber, K_2). The peak-to-peak noise is unchanged by the presence of the temperature control system, and the baseline is stable despite current swings of over 6 A through the Peltier devices.

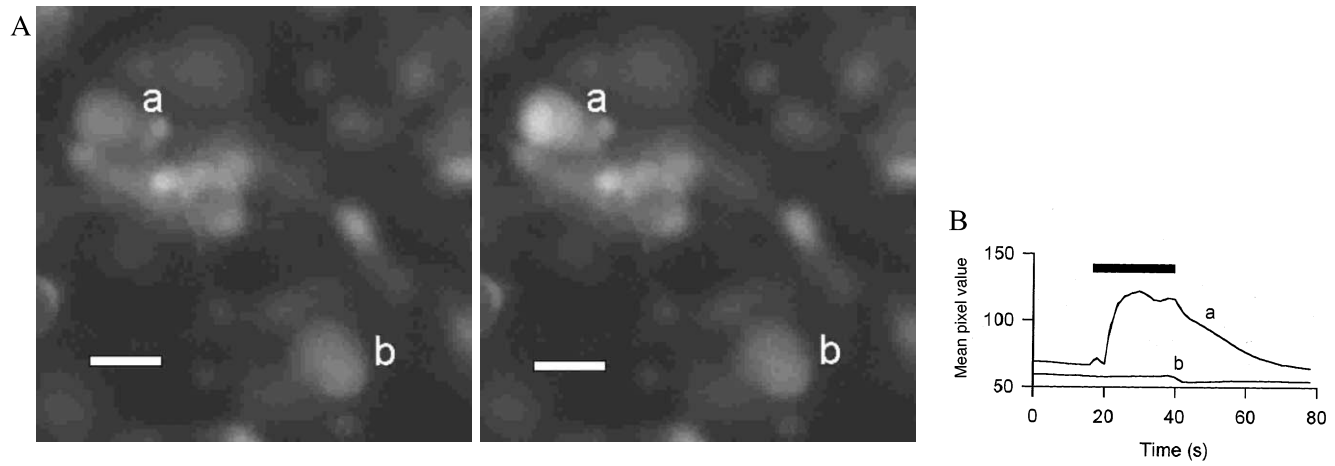

Fig. 5. Measurements of intracellular calcium ($[Ca^{2+}]_i$) during cooling stimuli applied with the system described here. DRG cultures were loaded with Calcium Green-1 AM in 0.02% Pluronic F-127 (Molecular Probes, Leiden, the Netherlands) for 30 min at 37 °C, then imaged with a Cohu 4910 CCD camera (Cohu Inc., San Diego, CA, USA) on a Nikon TE200 microscope (× 40, N.A. 0.95 dry objective) using software written by GR. Images were integrated for 1.6 s and acquired at 2 s per frame. (A) Images recorded from the same group of cells before (left) and during (right) a cooling ramp from 32 °C to 18 °C over a period of 22 s. The neurone marked 'a' responded to cooling with an increase in $[Ca^{2+}]_i$, while that marked 'b' was unresponsive. Scale bar 20 μm. (B) Mean pixel values (background subtracted) for the two cells indicated in A. The thermal stimulus was applied during the period marked by the bar.

power supplies would probably be a suitable alternative, but we have not been able to test this possibility on grounds of cost. As mentioned above, the chamber is shielded from the Peltier elements by thin grounded aluminium foil to reduce capacitive coupling of any remaining current noise and of the large current swings at the beginning and end of a temperature stimulus. Finally, the solution reservoirs and tubing are screened with grounded aluminium foil. Fig. 4C shows that with these precautions, the system does not increase the peak-to-peak noise recorded with a closed patch pipette in the recording bath, and the baseline current is stable despite large current swings through the Peltier elements.

4.2. Flow resistance and temperature effects on viscosity

We have mentioned that the resistance to solution flow in the heating/cooling chamber and manifold are low, and that we add an extra resistance element. The resistance introduced by this element should be much larger than that of the chamber, because it is important that the highest resistance in the system occurs at a point where the temperature of the solution is not changing. The reason for this is that the viscosity of water changes approximately four-fold over the temperature range from 10 °C to 50 °C. In the case of laminar flow through a round tube, the flow rate $\dot{V}$ is inversely proportional to the viscosity η according to the Hagen-Poiseuille equation:

$$\dot{V} = \frac{\pi r^2}{8\eta l}\Delta P \quad (2)$$

where:

r is the radius of the tube;

l is its length; and

ΔP is the difference in pressure along the tube.

If the highest resistance were in the chamber itself, the flow rate would therefore be very much faster at 50 °C than at 10 °C.

It is also useful if the resistance of the added resistance element is much higher than that of the manifold, for two reasons: firstly, small differences between the resistances of the separate tubes carrying each solution become insignificant and differences in flow rate between solutions are avoided; secondly, the flow rate does not depend on how many valves are open, allowing mechanical artefacts on change of solution to be avoided by starting flow of the new solution before the flow of the previous one is stopped.

4.3. Performance of Peltier devices and heat dissipation

Like any Peltier-based device, this system is somewhat more efficient in heating than in cooling (compare the upper and lower traces in Fig. 3A). This is because, while moving heat from one face to the other, the Peltier device also generates heat; this means that one face of the Peltier device is warmed a little more effectively than the other is cooled. Because the Peltier device is a heat pump, heat transferred from one side must be dissipated at the other side by an effective heatsink; if the rate of heat dissipation is not adequate, the whole device will begin to heat during a prolonged cooling stimulus. The cooling capacity of this system is therefore limited by the heat it generates, and by the efficiency with which this heat is removed. In extreme

cases, the heat generated during cooling can lead to a state of 'thermal runaway', where the device passes more and more current in an attempt to conduct away the heat that it is itself generating. This is a characteristic of all feedback controllers based on Peltier devices and in the present design is prevented by continuously monitoring the heatsink temperature and shutting down the controller if this exceeds a preset value (normally 60 °C).

In practice, the system presented here can heat well above ambient temperature (to at least 60 °C), and it can cool to about 5 °C below ambient for indefinite periods and 20 °C below ambient for brief periods ($\sim$15 s cool pulses, separated by $\sim$30 s) without additional measures to dissipate heat. To reach lower temperatures or to maintain low temperature for extended periods, a length of copper tubing (5 mm o.d.) is slotted into the heatsink and supplied by gravity with deionised water at or below room temperature (see Fig. 1B). This allows the chamber to be cooled to 15 °C below ambient indefinitely.

4.4. Effects of flow rate on temperature control

At high flow rates, the rate of heat transfer from the Peltier elements into the solution limits the maximum or minimum temperature that can be achieved at the cell. At low flow rates, heat transfer in the heating/cooling chamber is effectively complete, but heat exchange with the surroundings is a more serious problem. In order to determine the effects of flow rate on cell temperature and thus the optimum range of flow rates for the present system, we measured the temperature at the heating/cooling chamber (K_2 in Fig. 2), at the outlet of the chamber (T_1) and at the position of the cell (T_2) during step temperature commands from a base temperature of 32 °C at flow rates ranging from 0.6 to 1.7 ml/min. Bath temperature was 30 °C and feedback control was from thermocouple K_2.

Fig. 6A shows the effects of flow rate on static temperature for chamber temperatures of 0 and 50 °C; behaviour at other temperatures is essentially similar, but at command temperatures closer to ambient or bath temperature the errors are correspondingly smaller than shown here. At 0.6 ml/min, solution leaves the heating/cooling chamber within 1–3 °C of the command temperature, but its temperature changes by 5–7 °C by the time it reaches the cell. At the highest flow rate of 1.7 ml/min, the solution temperature changes by less than 1 °C between the outlet of the chamber and the cell, but at the cost of less than optimal heat transfer within the heating/cooling chamber. The optimum flow rate from the point of view of the final temperature reached at the cell is around 0.8–1 ml/min, and in this range, cell temperature does not depend sensitively on flow rate.

Higher flow rates are useful if rapid temperature changes are required, as shown in Fig. 6B for flow rates of 0.6, 1.0 and 1.7 ml/min. The temperature change at the cell is well described in each case by a single exponential function with a time constant of 5.8, 4.7 and 3.8 s respectively.

4.5. The recording bath

Because the system was designed for local temperature control, the design of the recording bath is not critical, but some comments based on our experience may be useful. Initially we used the system described here for patch clamp recording on cells grown in standard polystyrene 35 mm culture dishes. However, the large thermal expansion coefficient of polystyrene caused movement of the floor of the bath during large temperature steps, and this led to the loss of some whole cell patch clamp recordings. This problem is removed by using borosilicate glass coverslips (25 mm diameter, no. $1\frac{1}{2}$, 0.16–0.19 mm thick; Chance-Propper Ltd.) mounted in a commercially available Teflon coverslip holder (MSC-TD, Medical Systems Corp., Greenvale, NY, USA), with the coverslip fixed using silicone grease instead of the rubber O-rings supplied

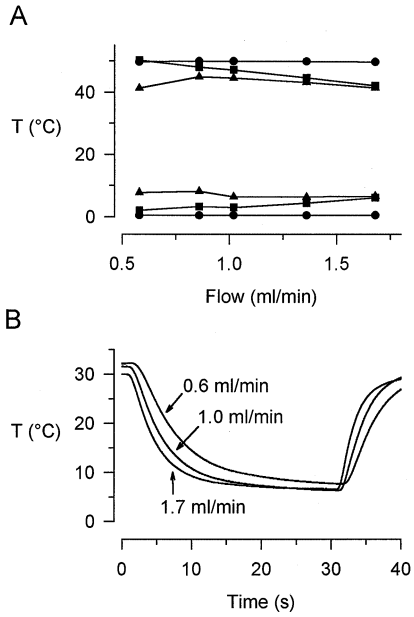

Fig. 6. Effect of flow rate on the static temperature and on the rate of temperature change. (A) Static temperature at the heating/cooling chamber (circles, measured at the thermocouple position shown in Fig. 1A with thermocouple K_2), at the outlet of the chamber (squares, thermocouple T_1) and at the cell (triangles, thermocouple T_2) at flow rates between 0.6 and 1.7 ml/min. Command temperatures were 50 °C and 0 °C and feedback was from thermocouple K_2. (B) Temperature at the cell during step temperature commands at flow rates of 0.6, 1.0 and 1.7 ml/min. The base temperature at the heating/cooling chamber was 32 °C and the command temperature was stepped to 0 °C for 30 s.

with the coverslip holder. We dispensed with these O-rings because their expansion or contraction during thermal stimuli tended to cause vertical movement and defocusing during imaging of intracellular [Ca^{2+}] (as well as instability during patch clamp recordings). We image with a dry objective (Nikon, 40 ×, N.A. 0.95) and condensation on the underside of the coverslip during strong cooling can disturb imaging experiments in warm, humid weather; this is easily prevented by directing a gentle stream of dry air at the underside of the coverslip.

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