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Activation of membrane receptors by neurotransmitter released from temperature-sensitive hydrogels

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Abstract

The present paper describes the design, construction and testing of a temperature-sensitive *N*-isopropylacrylamide hydrogel device for studying the controlled presentation of γ-aminobutyric acid (GABA) to $GABA_C$ membrane receptors expressed in *Xenopus laevis* oocytes. Upon temperature lowering, the GABA-loaded hydrogel positioned near the surface of the $GABA_C$-expressing oocyte elicits a membrane current response resembling that induced by superfusion of the oocyte with free GABA. The response to cooling is not observed when GABA is omitted from the hydrogel loading solution. In addition, picrotoxin, a known $GABA_C$ receptor antagonist, inhibits the oocyte membrane current response associated with temperature lowering of GABA-loaded hydrogels. The data indicate that the present system affords a temperature-regulated release of GABA from the hydrogel and a resulting activation of the expressed $GABA_C$ receptors.

Keywords: Hydrogel; Temperature-sensitive; GABA; Neurotransmitter; $GABA_C$ receptor

1. Introduction

The transmission of neural signals at chemical synapses involves the release of neurotransmitter by the pre-synaptic neuron and the interaction of this neurotransmitter with membrane receptor proteins of the postsynaptic neuron. Recent studies have investigated the use of microfluidic devices and chemical caging as approaches to the controlled delivery of neurotransmitter to retinal neurons (Peterman et al., 2003, 2004; Kapi et al., 2004). More generally, systems capable of presenting neurotransmitter to postsynaptic membrane receptors in response to an external stimulus may be useful for studying the fundamental physiology of synapses as well as for engineering applications.

Hydrogels are three-dimensional, water-swollen polymers that are crosslinked via covalent bonds, ionic interactions, hydrogen bonds or hydrophobic interactions (Peppas, 1997). Numerous recent studies have addressed the design and application of "smart" hydrogels, i.e., preparations that are responsive to controlled stimuli. For example, it is possible to control the swelling of the gels by changes in factors such as pH (Katchalsky, 1949; Mandracchia et al., 2004), temperature (Feil et al., 1992; Lee and Yeh, 1997; Ron and Bromberg, 1998; Zhang et al., 2003; Fisher et al., 2004; Liang et al., 2004), electric field (Jensen et al., 2002; Li et al., 2004) or light (Tanaka and Sato, 1972; Mamada et al., 1990; Qiu and Park, 2001). Such hydrogels are referred to as "stimulus-responsive hydrogels". The physical stimuli produce alterations in the molecular properties of the hydrogel that lead to changes in, e.g., swelling, solubility, macroscopic shape and crystalline/amorphous transitions (Iwata and Matsuda, 1988; Kim and Lee, 1999).

The properties of stimulus-responsive hydrogels raise the interesting question of whether these can be used to focally present neurotransmitter to postsynaptic membrane receptors. To investigate the feasibility of such stimulus-dependent neurotransmitter release from a hydrogel preparation, we have designed and tested a prototype system in which a temperature-sensitive, *N*-isopropylacrylamide (NIPAAm)-based hydrogel releases pre-loaded neurotransmitter in response to temperature

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lowering, i.e., in response to temperature-induced hydrogel swelling. As the test neurotransmitter and model postsynaptic cell we have used, respectively, γ-aminobutyric acid (GABA) (a major neurotransmitter of the central nervous system), and *Xenopus laevis* oocytes engineered to express ρ1 $GABA_C$ receptors (Qian et al., 1997, 1998). The ρ1 $GABA_C$ receptor represents a useful model system to test for neurotransmitter release. Beyond the relative structural simplicity of this receptor (homopentameric arrangement of the ρ subunits), $GABA_C$-mediated responses of this receptor activate and deactivate slowly, and typically exhibit little or no desensitization. When expressed in *Xenopus* oocytes, ρ1 $GABA_C$ receptors exhibit a robust response to GABA, and responses can be recorded for extended periods (Qian et al., 1997, 1998; Vu et al., 2005). Preliminary data were presented at the 2004 Biomedical Engineering Society meeting (Muni et al., 2004).

2. Materials and methods

2.1. Oocytes

All animal procedures were in accordance with institutional policies and with the Statement for the Use of Animals in Ophthalmic and Vision Research adopted by the Association for Research in Vision and Ophthalmology (ARVO). Adult female *X. laevis* were obtained commercially (Xenopus I Inc., Dexter, MI, USA) and maintained in aquaria at 20 °C. Animals were anesthetized with MS222 (3-amino benzoic acid ethyl ester; Sigma–Aldrich, St. Louis, MO, USA) (1.4 g/l). The ovarian lobes were excised; stage V and VI oocytes were removed using previously described procedures (Qian and Pan, 2002), and the oocytes were stored in saline solution (Ringer solution) containing 100 mM NaCl, 2 mM KCl, 2 mM $CaCl_2$, 1 mM $MgCl_2$, 5 mM HEPES, and 10 mM glucose, at pH 7.1–7.4. The oocytes were defolliculated by immersion in 2 mg/ml collagenase for 1 h at room temperature and by mechanical trituration using a large barrel glass pipette. Each oocyte was then injected (Drummond Nanoject II; Drummond Scientific Co., Broomall, PA, USA) with 50 nl (0.5 mg/ml) of cRNA that coded for the human $GABA_C$ ρ1 subunit; the cDNA source of the cRNA was generously provided by Dr. G.R. Cutting (Cutting et al., 1991). The oocyte was then incubated for 24–48 h at 16 °C in Ringer solution containing 0.1 mg/ml of gentamycin (Sigma–Aldrich).

2.2. Recording chamber and temperature control

Recordings of GABA-activated currents from a given oocyte were obtained after 1–3 days following cRNA injection. The oocyte (surrounded by Ringer solution) was positioned on a nylon mesh in the open well of a double-walled glass chamber (Fig. 1A). The volume of Ringer contained in this recording chamber was approximately 140–170 μl (depth of solution including the thickness of the mesh: about 2.5–3.0 mm). Unless otherwise indicated, the oocyte was superfused with Ringer at a rate of approximately 3 ml/min. In most experiments, the distance (3–5 mm) between the tip of the perfusion inlet tube and the surface of the solution in the chamber was bridged by a tissue paper wick, to reduce mechanical vibration associated with inflow of the perfusion medium. Temperature control of the Ringer solution within the well was achieved by circulating dry-ice-chilled ethanol (≈−50 to −25 °C) (Variable Flow Chemical Pump, Fisher Scientific, Hanover Park, IL, USA) through a compartment of the chamber that jacketed the Ringer- and oocyte-containing well. The hydrogel investigated in a given experiment was a cylindrical plug that was pre-loaded with GABA and Fast Green dye, and supported on the tip region of a silanized brass rod (see the following). The hydrogel-containing brass rod, mounted on a holder that in turn was held in a micromanipulator, was initially positioned above the surface of the Ringer solution surrounding the oocyte. A microscope (Leica Microsystems Inc., Bannockburn, IL, USA) equipped with a monochrome CCD camera interfaced with a computer afforded imaging of the preparation. A thermocouple sensor (Model CHAL-003, Omega Engineering Inc., Stamford, CT, USA) mounted near the floor of the well was used to record the temperature of the fluid within the chamber over the temperature range of 10–25 °C. The error determined for this calibrated thermocouple was approximately ±1 °C or less within the temperature range of 13–23 °C (Fig. 1B).

2.3. Collection and analysis of electrophysiological data

Membrane current responses of the oocyte were obtained using a two-microelectrode voltage clamp amplifier (GeneClamp500B; Axon Instruments, Foster City, CA, USA), and low-pass filtered at 10 Hz. The voltage-clamp procedure was controlled by a PC computer running Clampex 8.1 (Axon Instruments). Electrophysiological data were obtained in response to lowering the temperature of the chamber containing the oocyte and neighboring hydrogel; and in response to the presentation, to the oocyte, of free GABA and of picrotoxin (products of Sigma–Aldrich) dissolved in Ringer solution. The test solutions of GABA (10 μM) and picrotoxin (PTX; 100 μM) were delivered from separate reservoirs by a gravity flow system, controlled by a solenoid manifold and operated under computer command. Both the oocyte membrane current and the chamber temperature (see above) were simultaneously recorded by a computer system interfaced with the apparatus. The cells were voltage clamped at −70 mV and glass microelectrodes were filled with 3 M KCl. The pipette-to-bath resistance was typically 1–6 MΩ. The data collected were sampled at either 100 or 200 Hz by a DIGIDATA 1322A A/D board (Axon Instruments). Clampex 8.1 software (Axon Instruments) was used for data acquisition and Clampfit 8.1 (Axon Instruments) software together with SigmaPlot Version 8.0 (SYSTAT Software Inc., Point Richmond, CA, USA) and/or Origin Version 6.0 (OriginLab Corporation, Northampton, MA, USA) were used for the data analysis.

2.4. Hydrogel preparation and GABA-loading

2.4.1. Hydrogel composition

The GABA-delivery system was a NIPAAm-based temperature-sensitive hydrogel of composition similar to that used

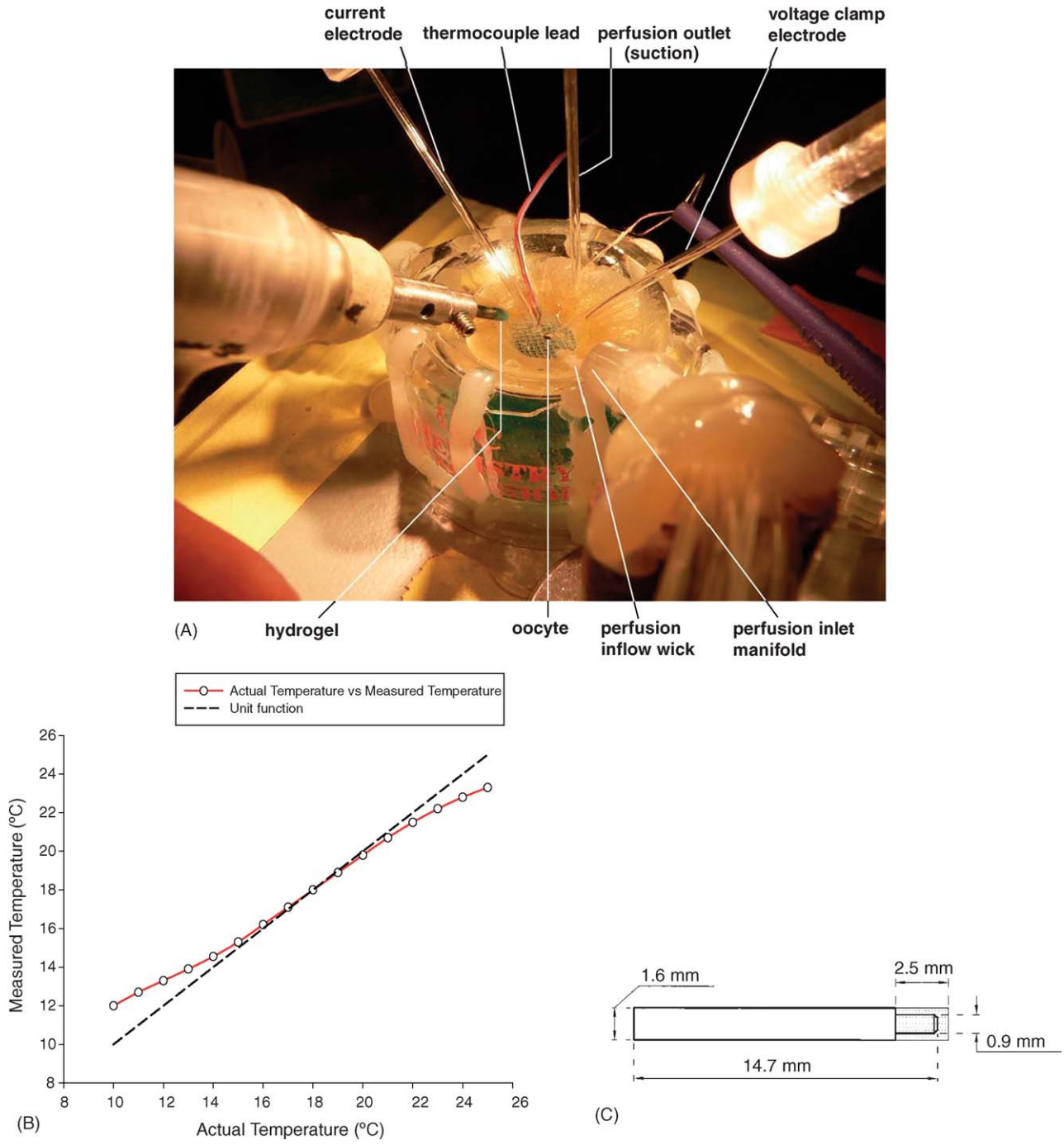

Fig. 1. Design and characteristics of recording chamber, and of the brass rod used to support the hydrogel. (A) Recording chamber, illustrating oocyte, electrodes for voltage clamp, and hydrogel. (B) Temperature calibration. Open circles show determinations of temperature reported by the thermocouple circuit (ordinate value) at a given bath temperature as measured by a calibrated thermometer (abscissa value). The dashed straight line is the unit function. (C) Schematic diagram of brass rod. Stipled region at the tip of the rod identifies the site of the hydrogel.

by previous investigators (Yoshida et al., 1994; Lee and Kim, 2003). For the preparation of the hydrogels, three stock solutions in ethanol–water (1:1, v/v) were prepared and stored at −20 °C in darkness. Solution 1 contained 50 mg/ml *N*,*N*′-methylenebisacrylamide; solution 2 contained 300 mg/ml *N*-isopropylacrylamide and 46.32 mg/ml of *N*-butoxymethylacrylamide (nBMA); and solution 3 contained 50 mg/ml 1-hydroxycyclohexyl phenyl ketone (all products of Sigma–Aldrich). The reaction mixture to be polymerized (see the following) was prepared in an amber-colored glass vial; the mixture, 800 μl in total volume, consisted of: 10.5 μl of solution 1; 462 μl of solution 2; 90.2 μl of solution 3; and 237.3 μl of ethanol/water (1:1, v/v). The final monomer mixture contained a mole ratio of 9 moles of NIPAAm to 1 mole nBMA, which was chosen based on preliminary studies (data not shown).

2.4.2. Holding rod

The investigated hydrogel preparation was formed as a small cylindrical plug on the tip region of a supporting brass rod. To facilitate adhesion of the hydrogel to the brass rod, a silane coupling agent was used as described previously (Guo and Gemeinhart, 2004; Kang et al., 2004). The rods were machined to the specifications shown in Fig. 1C. Each rod was then silanized by sequential washing in ethanol and dichloromethane, followed by immersion for 10–15 s in a freshly prepared solution of trichlorovinylsilane (Sigma–Aldrich) in dichloromethane (5:95, v/v). The rods were then sequentially rinsed in dichloromethane and ethanol, and then stored for 3 days at room temperature.

2.4.3. Hydrogel polymerization

For preparation of the poly(NIPAAm-co-nBMA) hydrogel coating of the brass tips, the brass rods (Fig. 1C) were positioned vertically (tip upward) on a circular base (Delrin®; diameter 2.5 cm, containing six evenly spaced, 1.6-mm diameter holes to accommodate the rods). Gels were formed around each tip using a mold consisting of Tygon tubing (i.d. = 1.6 mm, o.d. = 3.2 mm) that had been cut to a 7-mm length. Each Tygon section was cleaned with ethanol and then placed over the tip of a vertically positioned rod to form a tight seal with the thicker portion of the rod; the narrow space between the tubing and thinner portion of the rod defined the cylindrical mold to be occupied by the hydrogel (cf. Fig. 1C). The base holding the silanized brass rods fitted with Tygon tubing was then sealed in a plastic bag to exclude moisture. Both the sealed base assembly and a micro-syringe filled with the hydrogel mixture were separately stored at −20 °C for 1 h. Upon removal of the base assembly and micro-syringe from −20 °C storage, an aliquot (about 4 μl) of the hydrogel reaction mixture was immediately microinjected into the molds. Photopolymerization was carried out at 0 °C under UV illumination (365 nm) (Model EN-180 UV lamp; Spectroline, Westbury, NY, USA) for 3 min at 1.5 mW/cm^2. Immediately after the polymerization, the Tygon tubing was removed by sliding it downward over the base-end of the brass tip. For rinsing of the polymerized hydrogel and for incubation/storage of the hydrogel tip assemblies, a cylindrical plastic chamber (side wall and detachable roof) was secured to the base holding the hydrogel-tip assemblies, so that this base became the floor of a water-tight chamber (water-tight seal achieved by an *o*-ring surrounding the base). The assembled chamber was then filled with 50% ethanol/water and maintained at room temperature for ∼12 h to remove unpolymerized components from the hydrogel.

2.4.4. Hydrogel loading

The rinsing/incubation chamber containing the vertically positioned hydrogel-tip assemblies (see above) was used for introducing test solutions into the hydrogels. Unless otherwise indicated, the loading solution consisted of 100 mM GABA and 25 mM Fast Green Dye (Sigma–Aldrich); the latter component afforded visual inspection of the release of the loaded solution from the hydrogel. The loading treatment involved incubation of the hydrogel with GABA-dye solution overnight at 4 °C. This was followed by overnight storage at 36 °C, to collapse the hydrogel and trap the loaded GABA and dye. Loaded hydrogels were removed from the GABA-dye solution and gently rinsed with Ringer solution immediately before the experiment.

3. Results

3.1. Temperature characterization of hydrogel

Fig. 2A–C shows images of the hydrogel in the contracted and swollen states, while immersed in the Ringer solution in the recording chamber. The illustrated data were obtained at chamber temperatures of approximately 25, 19 and 16 °C, respectively. The volume transition temperature and degree of swelling were controlled by the composition of monomers and crosslinker to yield hydrogels with transition temperatures of about 15–20 °C. Fig. 2D illustrates the volume changes determined from a single hydrogel as the temperature of the bathing medium was varied over the range of 25–10 °C. Following each incremental change of temperature, the temperature of the bathing solution was allowed to stabilize at that test temperature for at least 30–40 s, a period determined in pilot measurements to be sufficient for near-equilibration of hydrogel swelling. An image of the immersed hydrogel at the test temperature was then captured. From the image of the hydrogel, the known mesh spacing (center-to-center distance of 0.95 mm) of the underlying nylon mesh, and the known inclination of the hydrogel-coated tip relative to the plane of the mesh, the outer dimensions and overall volume of the hydrogel-tip region were determined. To accomplish this, the shape of the plug was approximated as two co-axial cylinders with one cylindrical segment of narrower diameter and another cylindrical segment of greater diameter near the tip (see Fig. 2B and C). The volume of the encapsulated brass tip was then subtracted from this overall volume to yield the volume of the hydrogel plug. The swelling ratio was obtained from the relation, swelling ratio = $(V_T - V_{25})/V_{25}$, where V_T and V_{25} are the hydrogel volumes determined, respectively, at temperatures T and at 25 °C.

3.2. Temperature dependence of response to GABA-loaded hydrogel

Fig. 3A shows results from a representative experiment that tested the effect of temperature lowering of a GABA-loaded hydrogel positioned in proximity to the oocyte surface. The top trace is the membrane current as recorded by the voltage clamp. The middle trace, which is the instantaneous temperature recorded by the thermocouple, identifies the initial temperature of the recording chamber as 23 °C (i.e., room temperature). The event marker at the bottom of the figure indicates the hydrogel position, i.e., whether the hydrogel was in the vicinity of the oocyte or it was away from the oocyte and out of the recording medium. Unless otherwise indicated, Ringer solution continuously perfused the recording chamber at a rate of about 3 ml/min. The first phase of the experiment consisted of a 6-s presentation of Ringer supplemented with 10 μM GABA. This presentation of approximately 300 μl of 10 μM free GABA, which was

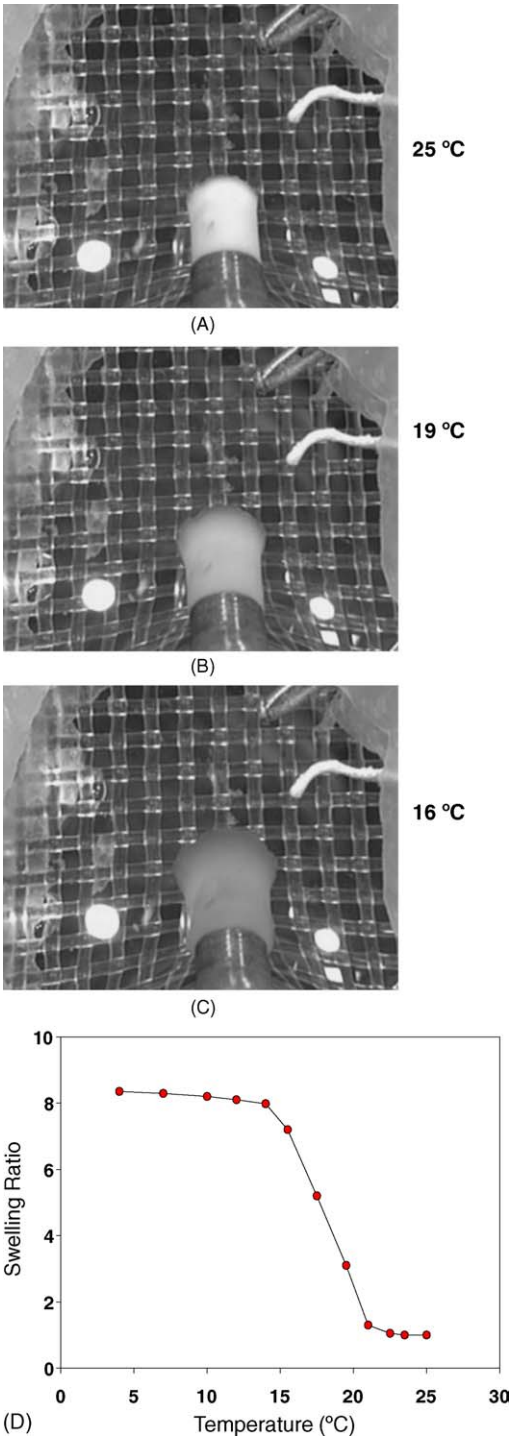

Fig. 2. (A) Image of hydrogel immersed in the recording chamber at approximately 25 °C. The nylon mesh within the chamber defined a grid of squares with center-to-center distance of approximately 0.95 mm. (B and C) Same hydrogel preparation, in the swollen state, at temperatures of approximately 19 and 16 °C, respectively. (D) Swelling curve. Swelling ratio for a representative (poly(NIPAAm-co-nBMA)) hydrogel as a function of temperature.

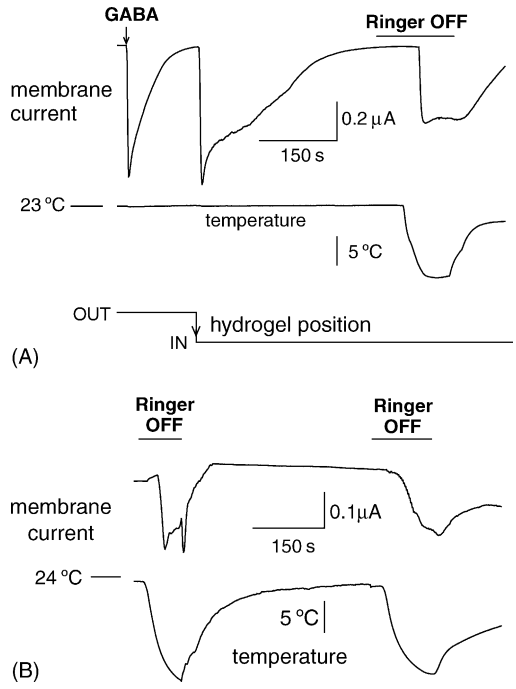

Fig. 3. Oocyte responses to GABA-loaded hydrogels. (A) Record obtained with, sequentially: the presentation of superfusing GABA (6-s pulse); initial immersion of a freshly loaded hydrogel (100 mM GABA and 25 mM Fast Green dye) in the Ringer solution at room temperature (23 °C); and subsequent lowering of the temperature of the chamber. Upper and lower traces indicate, respectively, membrane current of the oocyte and temperature of the recording medium. The event marker at the bottom of the figure indicates the position of the hydrogel as either withdrawn from the Ringer solution ("OUT") or immersed in the Ringer and near the oocyte ("IN"). (B) Results obtained from a different oocyte, with the hydrogel (pre-loaded with 100 mM GABA and 25 mM Fast Green dye) immersed in the Ringer solution throughout the experimental run, and with two rounds of temperature lowering. Format similar to that of the experiment shown in (A).

expected to produce a peak GABA concentration of at least several µM in the vicinity of the oocyte (chamber fluid volume: ≤170 µl; see Section 2), produced a robust membrane current response. Following recovery of the membrane current response to this control stimulation with free GABA, a freshly loaded hydrogel containing 100 mM GABA was gently lowered into the Ringer solution surrounding the oocyte, so that the hydrogel tip was ∼1 mm from the oocyte (see event marker). This initial presentation of the hydrogel to the vicinity of the oocyte at 23 °C led to a transient membrane current signal. This response was typical for initial immersion of the freshly loaded hydrogel, and was interpreted as a response to the leaching out of GABA at or near the hydrogel surface.

Upon recovery of membrane current to near baseline, the Ringer perfusion was turned off and cooling of the chamber was initiated (note the downward deflection of the temperature trace in Fig. 3A). The shut-off of Ringer perfusion during cooling was necessary to achieve rapid and extensive cooling. (In the absence of perfusion shut-off, the continuous infusion of room temperature Ringer overwhelmed the chamber cooling system and thus substantially diminished the effectiveness of cooling.) This cooling of the chamber, which produced a visually evident swelling of the hydrogel (cf. Fig. 2), elicited a membrane current response from the oocyte that was largely sustained throughout the period of lowered temperature. The re-initiation of perfusion with (room temperature) Ringer, which both washed out GABA released from the hydrogel and elevated the temperature of Ringer surrounding the hydrogel, led to a recovery of the

membrane current toward baseline. Upon raising of the temperature, a gradual de-swelling was also visually evident, although no attempt was made to correlate the de-swelling with either the kinetics of measured temperature rise or the recovery of the oocyte membrane current response.

Fig. 3B shows results from a second experiment in which the hydrogel was positioned near the oocyte membrane throughout the experimental run. Here, initiation of the illustrated trace corresponds with a time long after initial immersion of the freshly loaded hydrogel, and the transient response to initial immersion (cf. Fig. 3A) is not shown. As in the experiment of Fig. 3A, lowering of the temperature led to a membrane current response. Upon the re-initiation of Ringer perfusion and resulting temperature elevation, the membrane current recovered back to baseline. A subsequent lowering of temperature produced a similar membrane current response. Generally similar responses to temperature lowering were observed in five additional trials in the Fig. 3B experiment (data not shown). Repeated rounds of temperature lowering within a given experiment yielded responses of progressively diminishing peak amplitude. Typically, 5–7 rounds of temperature lowering reduced the peak response to about 50% of the maximal GABA response.

Fig. 4 shows results obtained in a control experiment in which the hydrogel was pre-loaded only with the Fast Green dye, i.e., GABA was omitted from the loading solution. As in the experiments of Fig. 3A and B, the hydrogel was immersed in the Ringer solution in proximity to the oocyte during the periods of temperature lowering, and temperature lowering was accompanied by a visually evident swelling of the hydrogel. Here, however, little if any change in membrane current occurred upon hydrogel cooling. Together, the results shown in Figs. 3A, 3B and 4 indicate that temperature lowering of the chamber elicits a membrane current response from the oocyte only when GABA is present in the hydrogel, i.e., that the oocyte response is elicited by released GABA.

3.3. Effect of picrotoxin

Picrotoxin (PTX) is an antagonist and a known suppressor of GABA-mediated responses at $GABA_C$ receptors (e.g., Qian et al., 2005). As a test of the hypothesis that the response to temperature lowering of the GABA-loaded hydrogel is mediated by the $GABA_C$ receptors expressed in the oocyte membrane, responses were obtained in both the absence and presence of PTX. Fig. 5A shows results of a representative experiment that tested the effect of PTX on the oocyte response to temperature lowering. In the first phase of the experiment, a response to temperature lowering of a GABA-loaded hydrogel was observed. Following recovery of that membrane current response, the hydrogel was withdrawn from the medium, and PTX (100 μM in Ringer solution) was perfused into the recording chamber as indicated by the horizontal bar. Removal of the hydrogel during this phase of the experiment prevented the entry of PTX into the hydrogel. Following the shut-off of PTX perfusion, the hydrogel was re-immersed in the recording medium close to the oocyte. Shortly thereafter, the temperature was lowered again. The response obtained to this lowering of temperature in the presence of PTX was smaller than that obtained in the

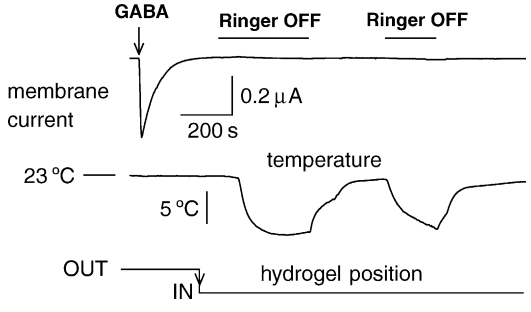

Fig. 4. Results obtained with the use of a hydrogel from which GABA was omitted (hydrogel pre-loaded with 25 mM Fast Green dye only). The record illustrates the response of the investigated oocyte to the presentation of free GABA (10 μM, 6-s pulse) and to temperature lowering of the preparation; format similar to those of Fig. 3A and B. Note from the hydrogel position marker that the hydrogel was immersed in the Ringer near the oocyte throughout the period that followed the response to the superfusing GABA.

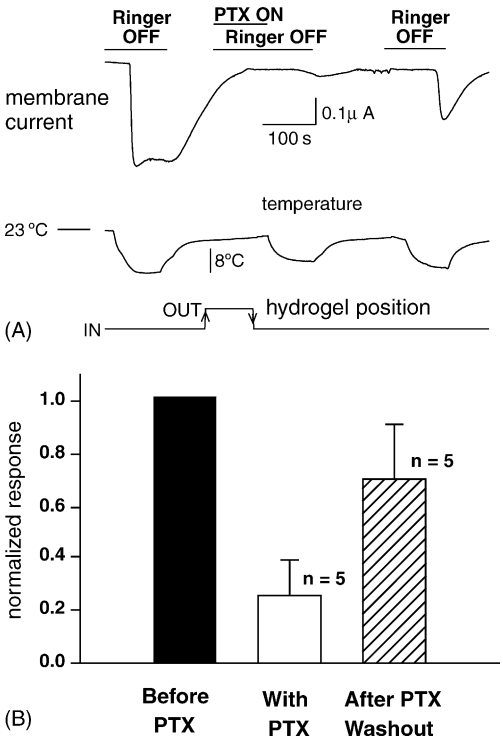

Fig. 5. Effects of picrotoxin. (A) Results from a single experiment showing responses to, sequentially: the lowering of temperature of a GABA-loaded hydrogel (100 mM GABA and 25 mM Fast Green dye) positioned near the oocyte; the infusion of 100 μM picrotoxin (PTX) and subsequent lowering of temperature; and temperature lowering following washout of the picrotoxin. As in Fig. 3B the membrane current and temperature records obtained in the initial phase of the experiment (initial immersion of freshly loaded hydrogel) have been omitted. (B) Histographic summary of results obtained in a total of five experiments (on four different oocytes) similar in design to the experiment of panel A. In all but one experiment, the investigated hydrogels were pre-loaded with 100 mM GABA and 25 mM Fast Green dye; in one experiment, the Fast Green dye was omitted. For each experiment, the peak response of the oocyte to temperature lowering determined before treatment with picrotoxin was taken as the normalizing amplitude; this peak amplitude is represented by the filled bar. Open and shaded bars represent normalized responses (average peak amplitudes) to temperature lowering in the presence of (open bar) and following wash-out of (shaded bar) 100 μM picrotoxin.

initial phase of the experiment, when PTX was absent. PTX was then washed out of the recording chamber and replaced by fresh Ringer solution. Subsequent lowering of the temperature led to a membrane current response considerably larger than that observed in the presence of PTX, indicating a substantial recovery from the action of PTX. It is evident from Fig. 5A that there was a relatively large delay between the onset of the final cooling episode and the onset of an inward current. It is possible that this delay resulted from a slower/smaller degree of cooling relative to the first episode. As the amplitude and kinetics of the temperature cooling during this final cooling episode did not differ dramatically from those of the first cooling episode, it is alternatively possible that the delay in onset of the membrane current response reflected long-lasting PTX inhibition and/or a reduction in the amount of GABA remaining available for release. These alternative possibilities are consistent also with the reduced amplitude of the response seen with the latter cooling.

Fig. 5B summarizes the results obtained in the experiment just described and in four others of similar design. The filled bar is the average peak temperature-stimulated response obtained before PTX introduction; the open bar indicates the average peak response upon temperature stimulation of the hydrogel in the presence of PTX; and the shaded bar shows the temperature-stimulated GABA response after PTX washout. Among the five experiments, the peak amplitude of the response during PTX treatment was 0.25 ± 0.13 (mean $\pm$ S.D.) that of the pre-treatment peak amplitude, and this decrease, as determined by pair-wise *t*-test, was significant ($P < 0.001$). Following washout of the PTX, the average peak amplitude was 0.69 ± 0.21 of that of the pre-treatment control, and *t*-test comparison of the data obtained during treatment versus after PTX washout indicated a significant recovery ($P = 0.004$). Together, the results shown in Fig. 5A and B indicate that PTX suppresses the response elicited by temperature lowering of GABA-loaded hydrogels.

4. Discussion

The present study describes a model system in which a chemical neurotransmitter (GABA) pre-loaded in a NIPAAm hydrogel is presented in temperature-controlled fashion to a cell (oocyte) expressing $GABA_C$ receptors. The results show that the investigated system elicits a membrane current response from oocytes expressing $GABA_C$ receptors, and that this response is suppressible by picrotoxin, a known $GABA_C$ receptor antagonist. From these findings we conclude that the temperature-responsive hydrogel employed here is suitable for physiological investigations of the expressed $GABA_C$ receptors.

A limiting factor in the interpretation of the GABA sensitivity at a given temperature concerns the geometry of GABA release from the hydrogel. The hydrogel is not a point source, but rather a cylindrical volume that presumably releases GABA in a complex manner that depends on factors such as the microscopic local permeability of the gel matrix, and the diffusion of released GABA around the oocyte. In light of these considerations, the present system is unable to address in quantitative fashion the local concentration of released GABA at the oocyte membrane.

To our knowledge, the temperature dependence of the GABA-mediated activation of $GABA_C$ ρ1 receptors has not been directly investigated. However, Borea et al. (1998), in an investigation of $\alpha_1\beta_2\gamma_2$ $GABA_A$ receptors, examined the temperature dependence of GABA-mediated receptor activation and found, over the range of 10–30 °C, that the EC_{50} for GABA increased by about 20%. Assuming a comparable EC_{50} change for GABA in the present experiments, one would expect the lowering of temperature from about 25 °C to about 13–15 °C to increase the apparent activity of GABA (i.e., to decrease the EC_{50}), by no more than about 10–20%.

A noteworthy feature of the data is the apparent delay between the initiation of the temperature drop and the development of the membrane current response (see, e.g., Fig. 3A). A likely factor underlying this delay follows from the visual observation that upon temperature lowering, Fast Green dye appeared only gradually in the medium surrounding the hydrogel. Assuming that this gradual rate of diffusion of the dye from the hydrogel corresponded roughly with the rate of diffusion of GABA from the hydrogel, this slow rate of GABA diffusion presumably contributed to the observed delay in the development of the oocyte response. Another possible basis for the observed delay is the placement of the temperature probe (i.e., thermocouple) within the recording chamber. The thermocouple was positioned on the nylon mesh near the floor of the Ringer-containing chamber, while the hydrogel was positioned ~1.5–2 mm above the floor of the chamber. It is therefore likely that the local temperature sensed by the thermocouple during the temperature-lowering procedure decreased more rapidly than the temperature of the medium immediately surrounding the hydrogel.

A further factor that contributes to the presently observed delay in the membrane current response is a relatively slow intrinsic responsiveness of the hydrogel (Li and Tanaka, 1990). However, temperature sensitive hydrogels with much faster response rates can be produced. For example, the introduction of microporosity (Kato and Takahashi, 1997), macroporosity (Gemeinhart et al., 2000), comb polymers (Yoshida et al., 1995), or polymeric grafts (Kaneko et al., 1998) can reduce the thermal response time. As an alternative to accelerating the intrinsic response rate, one can miniaturize the hydrogel, thus reducing both the mass of material that must undergo transition and the thermal transit distance (Li and Tanaka, 1990).

In conclusion, the present findings indicate the workability of the present system as a model for the stimulus-controlled, hydrogel-mediated presentation of neurotransmitter to functioning membrane receptors, and suggest the further development of this model system for studies of neurotransmitter–receptor interactions. Immediate candidates for further investigations of this type are hydrogels whose swelling and de-swelling properties are controllable by electric current (e.g., Qiu and Park, 2001; Kim et al., 2004). For example, the achievement of suitable time resolution, neurotransmitter capacity and biocompatibility of an electro-responsive hydrogel as a neurotransmitter delivery device, and the interfacing of such a device with the postsynaptic membranes of neurons in situ, might afford the regulation of nerve cell activity by hydrogel electro-activation. A possible alternative application of an electro-responsive hydrogel in

a focal delivery device would be as a gate or switch, rather than as the reservoir for the test agent as in the present study. Previous investigators have described the construction and operation of hydrogel microactuators that exhibit a rapid (sub-second) gating action in response to electrical stimuli (Richter et al., 2003; Wallmersperger et al., 2004; Santulli et al., 2005), and whose gating property is based on the same thermal transitions of the hydrogel as those employed in the present study.

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