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OSCILLATORY CHLORIDE CURRENT EVOKED BY TEMPERATURE JUMPS DURING MUSCARINIC AND SEROTONERGIC ACTIVATION IN *XENOPUS* OOCYTE

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SUMMARY

1. Membrane currents were recorded from voltage-clamped oocytes of *Xenopus laevis*, during temperature jumps imposed by a heating light. Resting oocytes usually showed little response, but large oscillatory membrane currents developed in response to cooling steps applied during activation of 'native' muscarinic receptors.

2. Similar temperature jump (T_{jump}) currents were seen during activation of oscillatory chloride currents mediated by muscarinic acetylchloline (ACh), serotonin, glutamate and noradrenaline receptors, expressed in the oocyte following injection with messenger ribonucleic acid (mRNA) from rat brain. The T_{jump} response during muscarinic activation was selectively blocked by atropine, and that during serotonergic activation by methysergide. In contrast, the 'smooth' membrane currents elicited by nicotinic ACh, kainate and γ-aminobutyric acid (GABA) were not accompanied by T_{jump} responses.

3. Rapid cooling of the oocyte gave larger T_{jump} currents than a gradual cooling over a few seconds. The size of the T_{jump} current elicited by a fixed cooling step increased linearly with the preceding time of warming, becoming maximal at intervals greater than about 100 s.

4. The T_{jump} current was inward at a clamp potential of -60 mV and reversed direction at about -22 mV, which corresponds to the chloride equilibrium potential in the oocyte. In low-chloride solution the reversal potential was shifted to more positive potentials, but it was almost unchanged by changes in potassium and sodium concentration. The size of the T_{jump} current decreased as the membrane potential was made more negative than about -40 mV.

5. The period of oscillation of the T_{jump} current increased with decreasing temperature, following a Q_{10} of 3·15. Depolarization also caused a small increase in period.

6. The T_{jump} current was not abolished in calcium-free solution, or by addition of manganese or lanthanum to the bathing solution. However, it was abolished by intracellular injection of the calcium-chelating agent EGTA.

7. Intracellular injection of inositol 1,4,5-trisphosphate evoked an oscillatory membrane current, during which T_{jump} responses developed similar to those after muscarinic activation. Intracellular injection of calcium evoked a chloride current, but this was not accompanied by T_{jump} responses.

8. We conclude that the oscillatory currents evoked by temperature jumps arise from chloride channels activated by intracellular calcium. This calcium is probably mobilized from intracellular stores by inositol trisphosphate which is liberated as a result of activation of muscarinic receptors, and also receptors for serotonin and glutamate. The oscillatory nature of the T_{jump} currents suggests that the pathway between these neurotransmitter receptors and channel activation involves negative feed-back.

INTRODUCTION

Oocytes of *Xenopus laevis* possess muscarinic acetylcholine (ACh) receptors, that give rise to chloride currents which show a characteristic oscillatory time course (Kusano, Miledi & Stinnakre, 1982). In addition to these 'native' receptors, the oocytes can be induced to acquire a wide variety of other neurotransmitter receptors, by injecting them with messenger ribonucleic acid (mRNA) from various sources (Barnard, Miledi & Sumikawa, 1982; Miledi & Sumikawa, 1982; Gundersen, Miledi & Parker, 1983, 1984*a*; Sumikawa, Parker & Miledi, 1984*b*). Activation of some of these exogenous receptors, including those to serotonin, glutamate, noradrenaline and dopamine, gives rise to oscillatory chloride currents, which appear almost indistinguishable from the native muscarinic responses (Gundersen *et al.* 1983, 1984*a*; Sumikawa *et al.* 1984*b*).

The oscillatory membrane currents elicted by all these agonists are usually irregular and 'spiky', although sometimes a more regular cyclic oscillation can be seen following a brief application of agonist (e.g. Fig. 1*B*, Miledi, Parker & Sumikawa, 1982*a*; Fig. 3, Gundersen *et al.* 1984*a*). However, we had previously noted that certain stimuli, including a hyperpolarization of the membrane, and a change in the rate at which fluid flowed past the oocyte, were able to synchronize the irregular agonist-induced current oscillations (Parker, Gundersen & Miledi, 1985*a*). In the present paper we report that rapid cooling of the oocyte, during activation of oscillatory drug-induced currents, elicits large oscillations in membrane current. These oscillations are generated by chloride currents, are dependent upon intracellular calcium, and can be induced in the absence of agonists by intracellular injection of inositol trisphosphate. Their appearance suggests that the internal messenger system which activates chloride channels in the oocyte membrane probably includes an element of feed-back, which can be easily perturbed by temperature changes.

METHODS

Most experiments were made on oocytes of *Xenopus laevis* which showed appreciable 'native' muscarinic responses to ACh. Other experiments used oocytes which had been injected a few days previously with mRNA from one of various sources (*Torpedo* electric organ, rat brain, or chick optic lobe) to induce the appearance of exogenous drug-activated membrane channels (Barnard *et al.* 1982; Miledi *et al.* 1982*a*; Miledi & Sumikawa, 1982; Gundersen *et al.* 1983, 1984*a*). Membrane currents were recorded from voltage-clamped oocytes, which were continually perfused with Ringer solution (Kusano *et al.* 1982; Miledi, 1982). The temperature of the solution was monitored by a thermocouple placed just down-stream of the oocyte, and unless otherwise stated the temperature was 24–26 °C. Normal Ringer solution contained 120 mM-NaCl, 2 mM-KCl, 1·8 mM-$CaCl_2$ and 5 mM-HEPES at pH 7·2. Zero-calcium Ringer solution contained no added calcium, and ad-

ditionally 2 mM-$MgCl_2$. Low-chloride solutions were made by partially replacing NaCl by Na methylsulphate. Isotonic KCl solution contained 120 mM-KCl in place of NaCl. Injections into the ooctye were made through a third micropipette, using either ionophoresis or pneumatic pressure ejection, as described previously (Miledi & Parker, 1984).

Rapid changes in temperature of the oocyte were effected by focusing light from a 100 W halogen microscope illuminator onto the oocyte. No heat filters were placed in the light path. Oocytes were positioned with their animal (black) hemispheres facing the light to maximize heat absorption, and also because the animal hemisphere has the greater density of calcium-activated chloride channels (Miledi & Parker, 1984). Illumination was controlled by manually interposing a vane into the light path, and a photodiode was used to monitor the light incident on the oocyte. Two methods were used to estimate the temperature rise produced in the oocyte by the heating light. The first was to replace the oocyte by a thermocouple, coated in black lacquer, which was of similar size to the oocyte. The second method was to record the small changes in 'leakage' current produced by the light in voltage-clamped oocytes, and to compare this with changes in current produced by different temperatures of perfusion solution. Both methods indicated that the light increased the oocyte temperature by about 2–3 °C, with the Ringer solution flowing past the oocyte at the rate normally used.

RESULTS

Membrane currents induced by cooling

Membrane currents were recorded from voltage-clamped oocytes in response to small but rapid changes in temperature, produced by interrupting the heating light focused onto the oocyte. When perfused with normal Ringer solution, most oocytes gave only small currents of a few nanoamperes in response to warming or cooling (e.g. first light change, Fig. 1*A*). These currents changed smoothly to a new steady level within a few seconds of turning the light on or off, and in the present experiments were in an outward direction (at −60 mV) following a cooling step. The outward currents on cooling probably arose from a decrease in resting 'leakage' conductance, and oocytes which had a low input resistance gave larger responses. However, on cooling, some oocytes recorded inward currents, which could be blocked by ouabain, and appeared to be due to a decrease in Na^+–K^+ pump activity at lowered temperature (R. Miledi & I. Parker, unpublished data). Occasionally, oocytes perfused in normal Ringer solution gave oscillatory currents on cooling, like those described below following drug activation. These responses were most prominent shortly after impalement of the electrodes. The experiments in the present paper were done using oocytes which failed to show these responses in the absence of agonists.

In contrast to the relative lack of temperature sensitivity in resting oocytes, large membrane currents were elicited when the same temperature steps were applied during muscarinic activation of the oocytes (Fig. 1*A*). Interruption of the heating light, and therefore cooling of the oocyte, caused inward oscillatory membrane currents, which grew progressively larger throughout several minutes of drug application. These responses will be referred to as temperature jump (T_{jump}) currents. ACh directly elicited an oscillatory membrane current, which in the oocyte of Fig. 1*A* was due to activation of muscarinic receptors induced following injection of mRNA from rat brain (Sumikawa, Parker & Miledi, 1984*a*). However, this current was smaller than that induced by the temperature jumps, and while it reached a fairly stable level within a minute of onset, the T_{jump} currents continued to increase for several minutes.

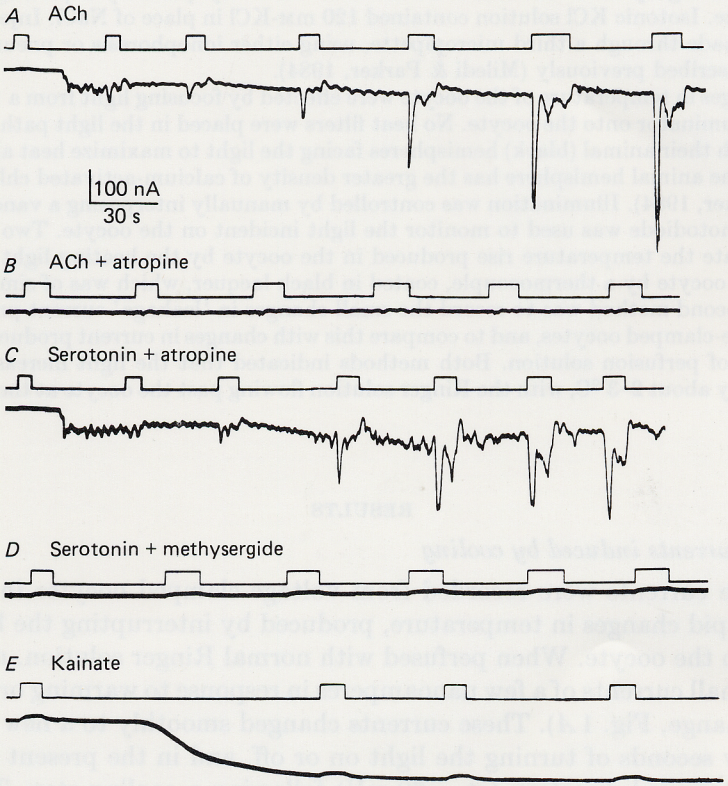

Fig. 1. Membrane currents recorded from voltage-clamped oocytes in response to temperature steps during application of different agonist drugs. Traces in *A–D* are from an oocyte injected with rat brain mRNA, and in *E* from an oocyte injected with chick optic lobe mRNA. The upper trace in each record monitors the heating light incident on the oocyte (down = on), and the lower trace shows membrane current. Downward deflexions of this trace correspond to inward membrane currents. Oocytes were clamped at −60 mV, and agonist drugs were applied by bath perfusion, beginning after the initial temperature step in each record. *A*, membrane currents during perfusion with ACh (10^{-6} M). *B*, application of ACh (10^{-6} M) together with atropine (10^{-7} M). Very small T_{jump} responses are visible during this trace, which occurred because an insufficient recovery time was allowed after obtaining the record in *A*. *C*, responses during perfusion with serotonin (10^{-7} M) plus atropine (10^{-7} M). *D*, application of serotonin (10^{-7} M) together with atropine (10^{-7} M) and methysergide (10^{-7} M). In *B–D*, perfusion with the blocking drugs (atropine and methysergide) began a few minutes before the start of each record. *E*, application of kainate (10^{-4} M).

Responses depend on temperature changes, not light

The T_{jump} currents reported here were produced by changes in illumination, and might thus have arisen in response to light, rather than to the resulting changes in temperature. Several observations indicate that temperature changes were the important factor. (i) Interposing a heat-reflecting mirror (type 5740, Oriel Corporation) in the light path elicited currents like those seen when the light was completely blocked, although of about one-half the size. This mirror transmitted visible wave-lengths with little reduction, but would have attenuated the total heat from

the tungsten–halogen source by about 60% (manufacturers specifications). (ii) Lowering the temperature of the solution flowing past the oocyte elicited similar, although smaller currents to those produced by extinguishing the light. This reduction in response size is expected because the rate of cooling of the solution was slow, and the size of the T_{jump} current is strongly dependent on the rate of cooling (see later).

T_{jump} responses depend upon activation of specific receptors

Oocytes obtained from some donors show 'native' muscarinic responses to ACh (Kusano *et al.* 1982) and in all such oocytes examined, T_{jump} currents were seen during perfusion with ACh (10^{-7}–10^{-5} M, e.g. Fig. 2). Other donors yield oocytes with little or no sensitivity to ACh, and in these cases cooling steps applied during application of ACh gave small or no inward currents. However, oocytes from such donors could be induced to acquire muscarinic receptors by injecting them with brain mRNA Sumikawa *et al.* 1984*b*), and activation of these exogenous muscarinic receptors was accompanied by T_{jump} responses.

In addition to the induction of muscarinic receptors, oocytes can also be made to express a wide variety of neurotransmitter receptors. Nicotinic ACh receptors were expressed following injection of mRNA from *Torpedo* electric organ (Barnard *et al.* 1982), but T_{jump} responses were not seen, even with high (10^{-3} M) concentrations of ACh, which elicited large membrane current responses. Similarly, the smooth membrane currents elicited by kainate and GABA in oocytes injected with mRNA from rat or chick brain (Miledi *et al.* 1982*a*; Gundersen *et al.* 1984*a*) failed to show T_{jump} responses (e.g. Fig. 1*E*), even during drug applications (10^{-4} M-kainate and 10^{-3} M-GABA) lasting several minutes. In all these cases, cooling produced a small outward current, but there was no evidence of oscillatory inward currents like those shown in Fig. 1*A*.

Activation of receptors to serotonin, glutamate and noradrenaline, induced in the oocyte by mRNA from brain, give rise to oscillatory chloride membrane currents, which closely resemble the oscillatory muscarinic response (Gundersen *et al.* 1983, 1984*a*; Sumikawa *et al.* 1984*b*). Application of these agonists led to T_{jump} responses (e.g. Fig. 1*C*) similar to those seen during muscarinic activation. The responses to serotonin, glutamate and noradrenaline did not, however, arise because of activation of muscarinic receptors, since both the direct responses to the agonists and the T_{jump} responses remained after the muscarinic response had been blocked by atropine (Fig. 1*B* and *C*). Conversely, the serotonin-activated current and the associated T_{jump} current could be selectively blocked by the serotonin antagonist methysergide (Fig. 1*D*).

Properties of the T_{jump} current

Cooling of the oocyte during activation of muscarinic (and also serotonin, glutamate and noradrenaline) receptors gave rise to inward membrane currents (at −60 mV), which showed a striking time course resembling a damped sinusoidal wave (Fig. 2*A*). The amplitude of this response was strongly dependent upon the rate of cooling, and became much smaller when the heating light was gradually reduced over a few seconds (Fig. 2*B* and *C*), rather than being suddenly interrupted (Fig. 2*A*).

Oscillatory membrane current responses were seen only to cooling, and not to warming of the oocyte. When the heating lamp was turned on after an interval in the dark, the agonist-induced membrane current showed a transient decrease (Fig. 1 *A* and *C*). In some oocytes this decrease was sufficient to completely suppress the agonist-evoked response for several seconds (Fig. 3), so that the oscillations in

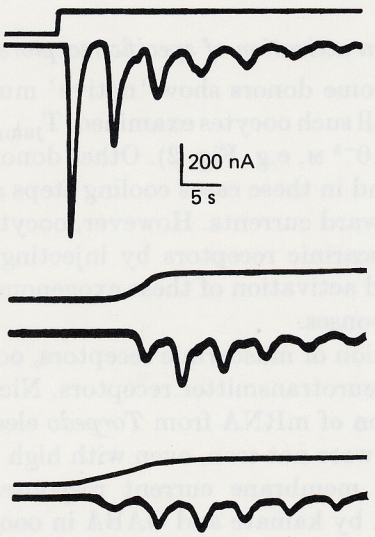

Fig. 2. Membrane currents elicited at different rates of cooling during ACh activation. In each frame the upper trace monitors the heating light (down = on), and the lower trace shows membrane current. Records from a native (non-mRNA-injected) oocyte, clamped at a potential of −60 mV. ACh (10^{-6} M) was applied for about 15 min before obtaining the records.

the current 'shut off', and the clamp current returned to the base line level observed before drug application.

During prolonged perfusion with ACh (10^{-6}–10^{-7} M) the size of the current evoked by a fixed cooling step increased progressively for 5 to 10 min (Fig. 1 *A*), even though the ACh-activated current often reached a peak and then declined during this time. Quantitative measurements of T_{jump} currents were made after exposing oocytes to ACh for at least 10 min, because the response size then remained fairly constant for many minutes. When the ACh was washed out, the size of the T_{jump} current declined more slowly than the direct ACh-activated current, and could still be elicited when the ACh current had ceased.

T_{jump} current is carried by chloride ions

T_{jump} currents elicited during ACh activation were inward at a clamp potential of −60 mV, but inverted direction when the potential was made more positive than about −20 mV (Figs. 4 *A* and 5 *A*). In ten oocytes (native and rat brain mRNA-injected) the mean equilibrium potential for the T_{jump} oscillations during muscarinic activation was $-22{\cdot}4 \pm 1{\cdot}1$ mV (mean ± S.E. of mean), which corresponds to the chloride equilibrium potential in the *Xenopus* oocyte (Kusano *et al.* 1982; Barish,

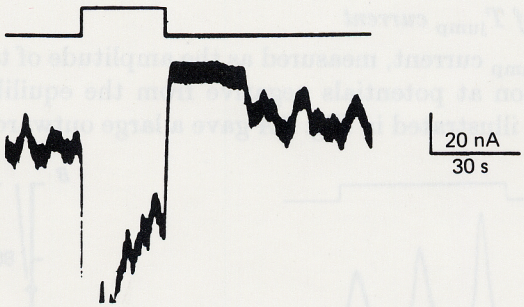

Fig. 3. Cessation of ACh-induced oscillatory currents on rewarming of the oocyte. Record from an oocyte injected with rat brain mRNA, clamped at −60 mV, and perfused with 10^{-7} M-ACh. The trace went off scale during the cooling step. Upper trace, heating light (down = on) and lower trace, membrane current.

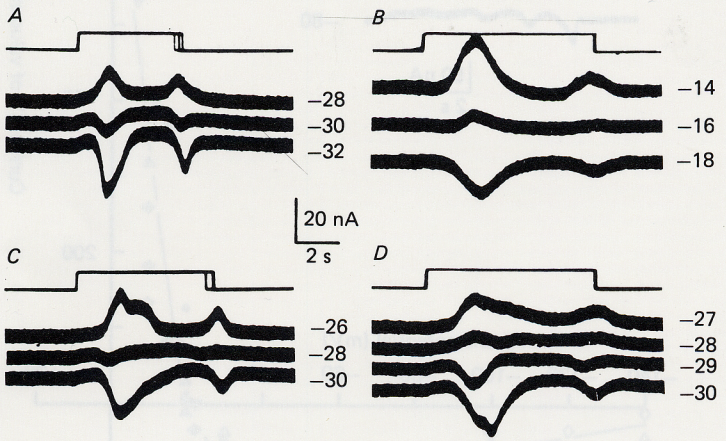

Fig. 4. Reversal potential of the T_{jump} current oscillations measured in one oocyte exposed to solutions of different ionic compositions. Each frame shows superimposed traces of membrane current obtained with the oocyte clamped at the potentials indicated (mV). The upper trace in each frame monitors the heating light (down = on). *A*, measurements in normal Ringer solution. *B*, shift of the reversal potential to a more positive value after replacing one-half of the chloride in the Ringer solution by methylsulphate. *C*, records in normal Ringer solution to which 18 mM-KCl was added to make the potassium concentration 20 mM. *D*, records in isotonic KCl solution (120 mM-potassium). The oocyte was returned to normal Ringer solution after each solution change, and the reversal potential determined in normal Ringer solution was −29 mV at the beginning of the experiment and −25 mV at the end. All solutions included 10^{-6} M-ACh. Oocyte had been injected with rat brain mRNA.

1983). A similar value (−19 mV) was obtained in one oocyte for the equilibrium potential of the T_{jump} response elicited during glutamate activation.

Further evidence indicating that the T_{jump} current is largely carried by chloride ions is that the reversal potential was shifted to more positive values in low-chloride (methylsulphate) Ringer solution (Fig. 4*B*), but was almost unchanged by increasing the potassium concentration from 2 to 20 mM (Fig. 4*C*), or by complete substitution of sodium by potassium (Fig. 4*D*).

Voltage dependence of T_{jump} current

The size of the T_{jump} current, measured as the amplitude of the first peak, showed a marked rectification at potentials negative from the equilibrium potential. For example, the oocyte illustrated in Fig. 5*A* gave a large outward T_{jump} current when

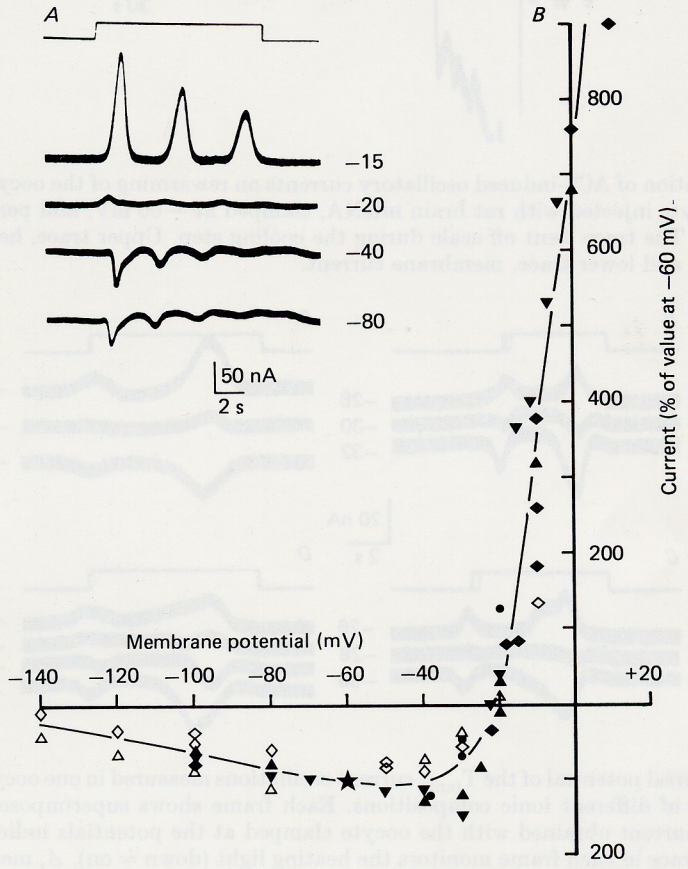

Fig. 5. Current–voltage relationship of the T_{jump} response. *A*, sample currents recorded from a native oocyte clamped at the potentials indicated (mV) during perfusion with 10^{-6} M-ACh. The heating light was turned off as indicated by the top trace (down = on). *B*, current–voltage relationship measured in seven oocytes (different symbols) from records similar to those in *A*. Points indicate the peak size of the first peak following the cooling step, normalized as a percentage of the response at −60 mV in each oocyte (marked by star). Curve is drawn by eye. All data from native (non-mRNA-injected) oocytes, perfused with 10^{-6} M-ACh.

clamped at 6 mV positive to the equilibrium, but a much smaller inward current when clamped 19 mV negative to the equilibrium. Furthermore, the size of the T_{jump} current actually declined when the potential was made increasingly negative beyond about −40 mV, despite the increased driving force for chloride efflux.

Fig. 5*B* shows the current–voltage relationship for the T_{jump} current elicited during ACh activation. At voltages more positive than the equilibrium potential the current

was steeply and monotonically graded with potential, but at more negative potentials the current reached a maximum at about −40 mV, and then declined with further hyperpolarization. The apparent conductance increase underlying the T_{jump} current at a potential of −140 mV was less than 1 % of that at 0 mV.

The period of the T_{jump} oscillations (measured as the interval between first and second peaks) showed a small voltage dependence, increasing with depolarization beyond about −50 mV (e.g. Fig. 5*A*). At −20 mV the period was increased by about 20 % of that at −60 mV, and at 0 mV it was about 50 % longer. However, no change was evident with alterations in potential between −60 and −100 mV.

Temperature dependence of period of oscillation

The period of the oscillations triggered by the T_{jump} became longer when the temperature of the perfusion fluid was lowered (Fig. 6*A*). Measurement of this effect was made by initially equilibrating the oocyte at a high temperature, and then taking readings as the temperature of the fluid was lowered. This was because the T_{jump} response was greatly reduced, or abolished, shortly after warming the solution, and recovered only after several minutes at the new temperature. On the other hand, cooling induced the appearance of oscillations in membrane current, so that the oocyte had to be maintained at a new temperature for a few minutes before a stable base-line was obtained.

Fig. 6*B* shows an Arrhenius plot of the period of the T_{jump} oscillations measured in three oocytes during ACh activation. The temperature indicated is that of the solution close to the oocyte, and would thus correspond to the equilibrium temperature of the oocyte when the heating light was off. Over the temperature range examined (14–27 °C) the period of oscillation followed a straight-line relation on the Arrhenius plot, with a slope corresponding to a Q_{10} of 3·15.

Recovery of T_{jump} current with different intervals of warming

The oscillatory current elicited by interrupting the heating light became smaller as the preceding interval of illumination was shortened (Fig. 7*A*). Very little response was detectable after warming for less than 10 s, but the current recovered to a maximal value after warming for about 2 min. The time course of recovery of the T_{jump} current measured from records like those in Fig. 7*A* is plotted in Fig. 7*B*. For periods shorter than about 100 s the current increased linearly with the time of warming, but failed to increase further with longer intervals.

A possible interpretation of this result could be that the time of recovery simply reflects the time of thermal equilibration of the ooctye. However, the oocyte (a sphere of about 1 mm diameter) is expected to warm up in a much shorter time than 100 s. Also, the fact that the size of the T_{jump} response was strongly reduced when the rate of cooling was slowed slightly (Fig. 2), suggests that the temperature of the oocyte adjusts within a few seconds after turning the heating light on or off.

Calcium dependence of the T_{jump} current

The oscillatory membrane currents activated in the oocyte by muscarinic and serotonergic receptors depend upon intracellular, but not extracellular, calcium (Parker, Gundersen & Miledi, 1985*b*; Dascal, Gillo & Lass, 1985). We were therefore

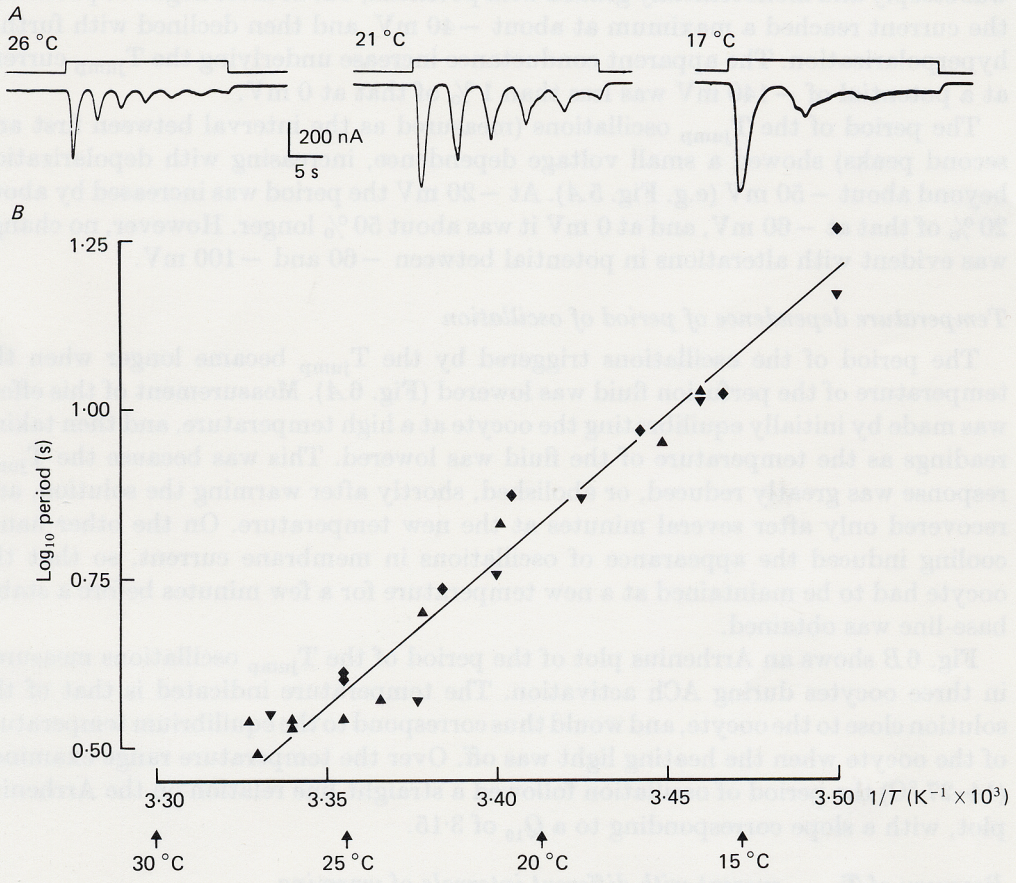

Fig. 6. Effect of temperature on the period of oscillation of the T_{jump} response. *A*, currents elicited in an oocyte perfused with Ringer solution at the different temperatures indicated. Oocyte was injected with rat brain mRNA, clamped at −60 mV and exposed to 10^{-6} M-ACh. Upper trace, heating light (down = on) and lower trace, membrane current. *B*, Arrhenius plot of the period of oscillation measured in three oocytes (different symbols). The period was measured from records like those in *A* as the interval between the first and second peaks of the oscillatory response, and the temperature (*T*) is that of the solution flowing past the oocyte. Line is drawn by eye.

interested to investigate the importance of calcium in the generation of the T_{jump} responses. For comparison with the T_{jump} responses, we also recorded the transient inward (T_{in}) current activated by hyperpolarization during muscarinic and serotonergic activation. This current is carried by chloride ions, but is dependent on an influx of external calcium (Parker *et al.* 1985*a*).

Recordings were made several minutes after beginning perfusion with ACh (10^{-6} M). At this time, hyperpolarization from −60 to −130 mV elicited a T_{in} current, followed by small oscillations in current, and an oscillatory T_{jump} current was seen on cooling (Fig. 8*A*). After changing to a Ringer solution containing no added calcium and 2 mM-magnesium the T_{in} current was almost abolished, but the T_{jump} current remained. In two oocytes the T_{in} current was reduced to less than 5 %

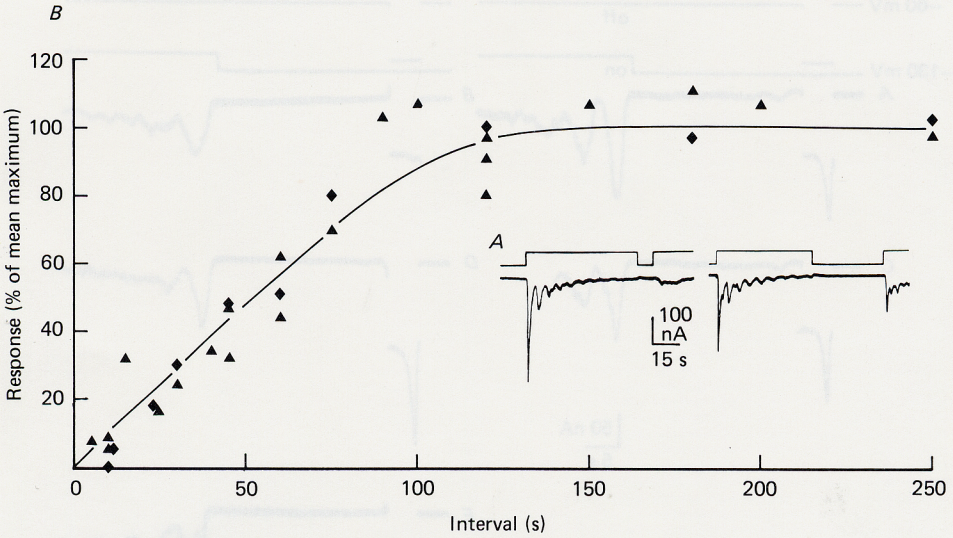

Fig. 7. Recovery of the T_{jump} response to cooling, following different preceding intervals of warming. *A*, membrane currents elicited by a cooling step, following different intervals of warming. The oocyte was exposed to the heating light for 180 s before the start of the first trace, and for 120 s before the second trace. Rat brain mRNA-injected oocyte, clamped at −60 mV and perfused with 10^{-6} M-ACh. *B*, data from two oocytes (different symbols), obtained from records similar to *A*. Responses were measured as the peak size of the first oscillation, and are normalized as a percentage of the mean maximal response obtained in each oocyte for intervals longer than 100 s.

of the control, while T_{jump} current was reduced to about one-half and showed less well synchronized oscillations than in normal Ringer solution. Increasing the calcium concentration from 1·8 to 10 mM enchanced the size of the T_{in} current (cf. Parker *et al.* 1985*a*), but slightly reduced the size of the T_{jump} current and it again became less synchronized (Fig. 8*C* and *D*). The mean size of the T_{in} current in three oocytes was enchanced in 10 mM-calcium to 150% of the control value, while the T_{jump} current was reduced to 76%.

Various 'calcium blocking' agents, including lanthanum, manganese and cobalt ions, are able to abolish or reduce the T_{in} current (Parker *et al.* 1985*a*). Addition of 1 mM-lanthanum to normal Ringer solution caused the T_{in} current to be completely abolished, but in contrast the T_{jump} current could still be elicited (Fig. 8*E*). Similarly, addition of 10 mM-manganese caused the T_{in} current to become barely detectable in two oocytes, while the T_{jump} current was enhanced in one oocyte, and slightly reduced in the other.

The role of intracellular calcium in the generation of the T_{jump} response was examined by injecting oocytes with the calcium-chelating agent EGTA. After loading with EGTA, the oscillatory membrane current directly activated by ACh was abolished (cf. Parker *et al.* 1985*b*; Dascal *et al.* 1985) as was the T_{in} current activated by hyperpolarization (cf. Parker *et al.* 1985*a*). Furthermore, no outward oscillatory T_{jump} currents were seen, even after prolonged (15 min) application of ACh in oocytes which gave large responses before loading (Fig. 9). Results similar to those illustrated were obtained in two other oocytes.

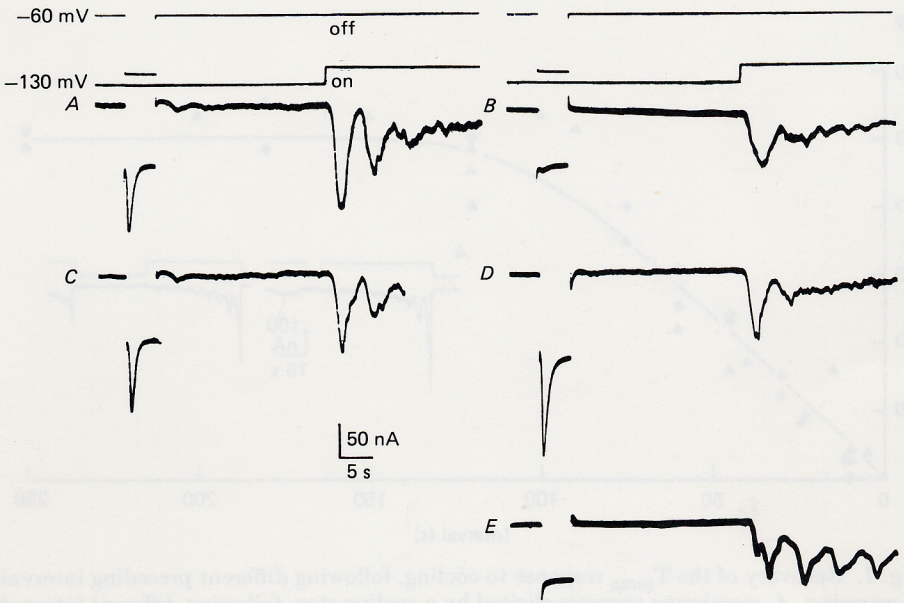

Fig. 8. Dependence of T_{in} and T_{jump} currents on external calcium. Current records show responses to a hyperpolarizing pulse from −60 to −130 mV, and to a cooling step (up = heating light off), as indicated by the top two traces. *A*, control records in normal Ringer solution. *B*, currents recorded a few minutes after beginning perfusion with Ringer solution containing 2 mM-Mg^{2+} and no added calcium. *C*, control record obtained a few minutes after returning to normal Ringer solution. *D*, enhancement of T_{in} current in Ringer solution containing 10 mM-calcium. *E*, record from a different oocyte, showing the presence of the T_{jump} response, but not the T_{in} current in a Ringer solution to which 1 mM-lanthanum was added. All solutions contained 10^{-6} M-ACh, and all records were obtained at a holding potential of −60 mV. The oocyte in *A–D* was injected with rat brain mRNA; that in *E* was native.

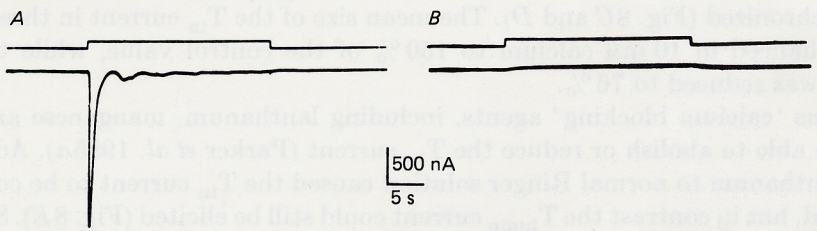

Fig. 9. Blocking of the T_{jump} oscillations by intracellular injection of EGTA. Upper trace, heating light (down = on) and lower trace, membrane current. *A*, control record obtained during perfusion with ACh (10^{-6} M). *B*, current recorded under similar conditions, but after loading the oocyte with EGTA using an ionophoretic current of 100 nA for 10 min. Native oocyte, clamped at −60 mV.

Intracellular injection of $InsP_3$ and calcium

Injection of inositol 1,4,5-trisphosphate ($InsP_3$) into *Xenopus* oocytes generated an oscillatory chloride current which mimicked the currents elicited by muscarinic and serotonergic activation (Fig. 10*A*; see also Oron, Dascal, Nadler & Lupu, 1985). This similarity between the $InsP_3$- and the drug-activated responses included also the

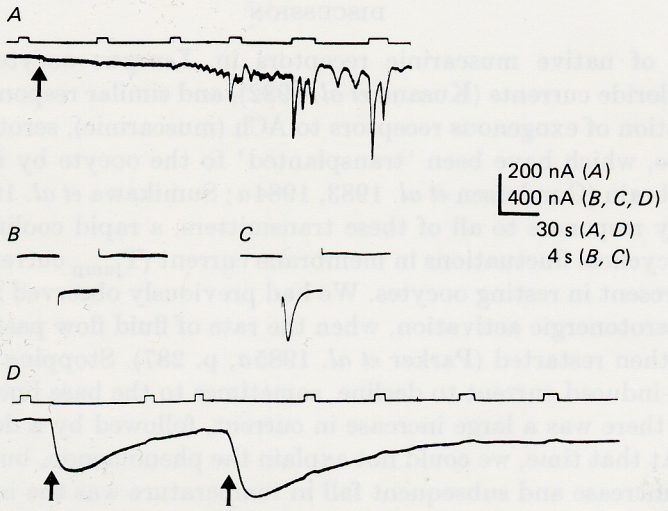

Fig. 10. Intracellular injections of $InsP_3$ (*A–C*) and calcium (*D*) into native oocytes. All records at a holding potential of −60 mV. Upper traces in *A* and *D* monitor the heating light (down = on). *A*, membrane currents in response to temperature jumps. At the arrow, a single pressure pulse (100 kPa, 200 ms duration) was applied to an intracellular pipette containing 1 mM-$InsP_3$. The volume of solution ejected by this pulse, estimated from the diameter of the droplet expelled with the pipette in air, was about 50 pl. This corresponds to 50 fmol of $InsP_3$, which would give an intracellular concentration of 50 nM, assuming even distribution throughout the oocyte. *B* and *C*, development of T_{in} current following $InsP_3$ injection. Both frames show membrane currents elicited by stepping the clamp potential from −60 to −120 mV during a pulse of 4 s duration. The record in *B* was obtained before, and that in *C* several minutes after, injection of three pulses of $InsP_3$ (same oocyte and injection parameters as in *A*). *D*, lack of response to temperature jumps following intracellular injections of calcium. Calcium injections were made as indicated by the arrows, using pressure pulses of 100 kPa for 200 ms, applied to a pipette containing 50 mM-$CaCl_2$.

appearance of T_{jump} and T_{in} currents following $InsP_3$ injection (Fig. 10*A*, *B* and C). The properties of the T_{jump} response following $InsP_3$ injection were similar to those during ACh application; the size of the current grew over several minutes after the injection, the time course of the oscillations was similar, the reversal potential measured in one oocyte was −21 mV, and the current showed a strong rectification at negative membrane potentials. Membrane currents elicited by $InsP_3$, and the associated T_{jump} responses, persisted for 10–20 min following injection, and could then be repeated by subsequent injections of $InsP_3$.

In contrast to the oscillatory currents evoked by $InsP_3$, injection of calcium ions into the oocyte usually generated a 'smooth' chloride current (Fig. 10*D*), although oscillations in current are sometimes seen following large calcium injections (Miledi & Parker, 1984; Dascal *et al.* 1985). Temperature jumps applied following calcium injections into the oocyte failed to elicit oscillatory responses, even with injections which evoked large membrane currents (Fig. 10*D*). However, application of ACh (10^{-4} M) to the oocyte illustrated in Fig. 10*D* caused the development of oscillatory outward T_{jump} currents, even though the direct ACh-activated current was much smaller (80 nA) than the calcium-activated current.

DISCUSSION

Activation of native muscarinic receptors in *Xenopus* oocytes gives rise to oscillatory chloride currents (Kusano *et al.* 1982), and similar responses are seen also during activation of exogenous receptors to ACh (muscarinic), serotonin, glutamate and dopamine, which have been 'transplanted' to the oocyte by injecting it with mRNA from brain (Gundersen *et al.* 1983, 1984*a*; Sumikawa *et al.* 1984*a*, *b*). During the oscillatory responses to all of these transmitters, a rapid cooling of the oocyte elicited large cyclical fluctuations in membrane current (T_{jump} currents), which were only rarely present in resting oocytes. We had previously observed responses of this kind during serotonergic activation, when the rate of fluid flow past the oocyte was stopped and then restarted (Parker *et al.* 1985*a*, p. 287). Stopping the flow caused the serotonin-induced current to decline, sometimes to the base line, and when flow was resumed there was a large increase in current, followed by a decaying series of oscillations. At that time, we could not explain the phenomenon, but it now appears that a slight increase and subsequent fall in temperature was the important factor.

The T_{jump} currents are carried largely by chloride ions, show rectification at negative membrane potentials, and depend upon intracellular, but not extracellular calcium. These properties closely resemble those of the oscillatory responses directly activated by muscarinic, serotonergic and other receptors, which are also due to chloride currents (Kusano *et al.* 1982; Gundersen *et al.* 1983), depend upon intracellular calcium (Parker *et al.* 1985*b*; Dascal *et al.* 1985), and rectify at hyperpolarized potentials (Kusano *et al.* 1982; Gundersen *et al.* 1983; R. Miledi, I. Parker & K. Sumikawa, unpublished data). Furthermore, we have previously shown that the membrane of native *Xenopus* oocytes contains chloride channels which can be activated by intracellular calcium, and these also show a similar rectification. It seems likely, therefore, that the same calcium-activated chloride channels may underlie the T_{jump} response, the oscillatory responses to agonist activation, the current evoked by intracellular injection of calcium (Miledi & Parker, 1984), and the transient outward current elicited by a depolarization-activated rise in intracellular calcium (Miledi, 1982; Barish, 1983; Miledi & Parker, 1984).

Chloride currents activated by exogenous GABA and glycine receptors expressed in the oocyte also rectify at negative potentials (Miledi *et al.* 1982*b*; Gundersen, Miledi & Parker, 1984*b*). These neurotransmitter responses do not depend upon intracellular calcium, and thus probably arise via chloride channels different to those discussed above. Rectification of the glycine-activated current appears to arise from a voltage-dependent shortening of open channel lifetime (Gundersen, Miledi & Parker, 1986), but we do not yet know if this mechanism applies more generally to other types of chloride channel.

Injection of $InsP_3$ into oocytes evoked oscillatory membrane currents resembling those mediated by muscarinic, serotonergic and other receptors (Fig. 10; see also Oron *et al.* 1985). This resemblance included also the development of T_{jump} responses, and of a transient inward current activated by hyperpolarization (Parker *et al.* 1985*a*). The similarities between agonist-evoked responses give strong support to the view that phosphoinositide and calcium metabolism may be the link between receptor activation and the generation of the oscillatory chloride current in the oocyte (Oron

et al. 1985; Parker *et al.* 1985*b*; Dascal *et al.* 1985). In this hypothesis, receptor activation results in the increased hydrolysis of phosphatidylinositol 4,5-bisphosphate to produce $InsP_3$, which then causes mobilzation of calcium from internal stores. The released calcium, in turn, activates the membrane chloride channels. This calcium-mobilizing activity of $InsP_3$ has been well documented in a variety of permeabilized cells (Berridge & Irvine, 1984), and has recently been domonstrated also in intact oocytes (Busa Ferguson, Joseph, Williamson & Nuccitelli, 1985; Parker & Miledi, 1986).

The damped sinusoidal oscillations in chloride current elicited by temperature steps suggest that the internal messenger system controlling the chloride channels may include a feed-back element, so that when perturbed, the system settles to a new equilibrium level following an oscillatory time course. The temperature dependence of the period of the oscillations is high ($Q_{10} = 3{\cdot}15$), which would indicate that the rate-limiting step is likely to be an enzymatic reaction, rather than, for example, the diffusion time of some substance within the oocyte. Further, the induction of T_{jump} responses following injection of $InsP_3$ into the ooctye suggests that the mechanism generating the oscillations can be activated independently of receptor binding and receptor-mediated hydrolysis of inositol phospholipids, and instead is limited to the train of events initiated by $InsP_3$. Injections of calcium ions into the oocyte, however, failed to induce T_{jump} responses. A calcium-induced release of calcium from intracellular stores (cf. Endo, 1977) is, therefore, unlikely to be the mechanism responsible for the oscillations. Instead, it may be that the mobilization of calcium by $InsP_3$ is regulated by the cytosolic free calcium level.

During fertilization, oocytes show changes in membrane potential which are accompanied by an increase in cytosplasmic calcium (see Whitaker & Steinhardt, 1982 for review). These changes can be mimicked by intracellular injection of $InsP_3$ (Whitaker & Irvine, 1984; Busa *et al.* 1985; Slack, Bell & Benos, 1986), suggesting that they involve processes similar to those which give rise to the oscillatory chloride current. Furthermore, cyclic oscillations in intracellular calcium have been observed in mouse oocytes following activation by sperm (Cuthbertson & Cobbold, 1985), so it is likely that the mechanism responsible for generating the oscillations is important during fertilization.

A similar oscillatory mechanism might also be present in neuronal cells, because exogenous receptors to ACh, serotonin and certain other neurotransmitters, transplanted to the oocyte from brain, are able to activate oscillatory currents almost identical to those seen with muscarinic activation in native oocytes. This probably occurs because they 'link in' to the mechanism already present in the oocyte, but we do not yet know whether these receptors give rise to similar responses in their native neuronal cells (Gundersen *et al.* 1983). However, it seems that similar internal messenger systems are sometimes involved. For example, muscarinic agonists (Nishizuka, 1984; Vicentini, Ambrosini, DiVirgilio, Pozzan & Meldolesi, 1985) and excitatory amino acids (Sladeczek, Pin, Récasens, Bockaert & Weiss, 1985) increase inositol phospholipid turn-over in neurones. The presence of an internal messenger system able to generate rhythmic oscillations could be of great importance for regulating neuronal excitability. Although abrupt temperature changes would not be expected in the mammalian brain, other more physiological stimuli might serve to

elicit synchronized activity. For example, regular oscillations can be seen in oocytes following rapid activation by ACh (Miledi *et al.* 1982*b*), as would occur during synaptic activation, and also following a brief influx of calcium into the cell (Parker *et al.* 1985*a*).

Cyclical oscillations in intracellular free calcium levels have been observed in various cells in addition to oocytes, including ventricular muscle (Allen, Eisner & Orchard, 1984), and hepatocytes (Woods, Cuthbertson & Cobbold, 1986). It will be interesting to see if any common mechanisms underlie all of these oscillatory responses.

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