

# Horizontal cell gap junctions: Single-channel conductance and modulation by dopamine

(retina/electrical coupling/voltage clamp/noise analysis)

D. G. McMAHON\*, A. G. KNAPP†, AND J. E. DOWLING

Department of Cellular and Developmental Biology, Harvard University, 16 Divinity Avenue, Cambridge, MA 02138

Contributed by J. E. Dowling, July 18, 1989

**ABSTRACT** Horizontal cells form an electrically coupled network for the transmission of inhibitory signals in the outer retina. In teleosts, horizontal cell coupling is modulated by the neurotransmitter dopamine. Using voltage-clamped pairs of teleost horizontal cells, we have examined the effects of dopamine on the conductance and gating properties of the cell-to-cell channels that mediate electrical synaptic transmission. Variance analysis of the junctional current noise showed that dopamine substantially reduced the open probability of gap junction channels, from 0.75 to 0.14. Direct observation of unitary junctional gating events in poorly coupled cell pairs indicated that these channels have a unitary conductance of 50–60 pS. The elementary conductance of channels in cell pairs treated with dopamine ( $48.7 \pm 6.6$  pS) was statistically indistinguishable from channels in untreated cells ( $53.2 \pm 7.2$  pS). Uncoupling with octanol also yielded a similar unitary conductance ( $61.1 \pm 11.1$  pS). Our results suggest that dopamine reduces the open probability of gap junctional channels by decreasing their open duration.

Intercellular communication at gap junctions is mediated by cell-to-cell channels. Evidence from a variety of biological systems suggests that these channels open and close in an all-or-none manner, similar to other membrane channels, and that permeability of gap junctions can be modulated by receptor-activated second messenger systems (1, 2). In the retina, the neurotransmitter dopamine decreases gap junctional permeability between horizontal cells via cyclic AMP (3–5). In other tissues, cyclic AMP also appears to regulate junctional permeability (6–8). Recently, the single channel physiology of non-neuronal gap junction channels has been described by incorporation of purified gap junctions (9), reconstitution of junctional proteins (9, 10), or pairwise voltage clamp of isolated cell pairs (11–13). We describe here the properties of neuronal gap junction channels from teleost horizontal cells.

The extensive electrical synapses between retinal horizontal cells mediate the lateral spread of inhibitory signals in the outer retina (14, 15). Dopaminergic modulation of these synapses reduces the size of horizontal cell receptive fields in fish and turtles (16–18) and limits dye coupling between horizontal cells (4, 18). Dopamine acts on horizontal cell coupling through a cyclic AMP-dependent mechanism; dopamine receptors in carp and white perch horizontal cells are coupled to adenylate cyclase (19, 20). In addition, cyclic AMP analogues mimic the uncoupling effects of dopamine in intact retinas (3, 4) and on isolated pairs of teleost horizontal cells (21). Furthermore, in teleosts, dopamine and cyclic AMP act through protein phosphorylation. Intracellular injection of protein kinase inhibitor blocks uncoupling by

dopamine, while injection of kinase A catalytic subunit mimics the effects of dopamine (22).

Whereas significant progress has been made in elucidating the biochemical steps involved in the regulation of horizontal cell junctional permeability, little is known about the functional properties of the junctional channels and how they are modified by dopamine and cyclic AMP. Dopamine might reduce the macroscopic junctional conductance through three kinds of effects, by reducing (i) the number of functional channels, (ii) the elementary conductance of individual channels, or (iii) the open probability of individual channels. We have examined dopamine's action on the junctional channels of white perch horizontal cells by analysis of current noise and of single channel events. Our findings indicate that horizontal cell gap junction channels have a unitary conductance of 50–60 pS and that dopamine primarily affects the gating kinetics of junctional channels rather than their number or elementary conductance.

## METHODS

**Cell Culture.** Pairs of electrically coupled horizontal cells in primary cell culture were obtained by mechanical dissociation of papain-treated white perch (*Roccus americana*) retinas (23). After dissociation, cells were maintained in L-15 medium (GIBCO) at room temperature (20°C). Recordings were made on cells after 2–10 days in culture. In most experiments, pairs of the small H1 cells were used to minimize the contribution of extrajunctional membrane to the recordings.

**Recording.** The whole-cell patch clamp technique for recordings was used (25). Each cell of an electrically coupled pair was individually voltage clamped. Pairwise voltage clamp is discussed extensively elsewhere (11, 12). Electrode resistance measured in the bath was 5–15 M $\Omega$ . Series resistance, which generally ranged from 5 to 20 M $\Omega$ , was monitored by simultaneous –20-mV pulses applied to both cells and was electronically compensated. As in previous studies, the extrajunctional resistance of most horizontal cells, measured in this case with both cells of the pair clamped at the same potential, was in the 0.5- to 1-G $\Omega$  range (21). The output of each amplifier was filtered at 1 kHz (–3 decibels), digitized at 44.1 kHz, and stored on VCR tape. Sections chosen for analysis were redigitized at 2 kHz per channel using PCLAMP software (Axon Instruments, Burlingame, CA). For analysis of unitary events, data were refiltered at 250 Hz during redigitization. Only events of sufficient duration to allow filter settling ( $\geq 6$  msec) were used in constructing amplitude histograms. Open duration was determined as the average duration of all channel openings that exceeded a half-level threshold. Noise analysis was performed with in-house software, while unitary event analysis was accomplished with

The publication costs of this article were defrayed in part by page charge payment. This article must therefore be hereby marked "advertisement" in accordance with 18 U.S.C. §1734 solely to indicate this fact.

\*To whom reprint requests should be addressed.

†Present address: Cambridge Neuroscience Research, Inc., Cambridge, MA 02139.

PCLAMP. For 7 of the 10 pairs used in noise analysis, the mean and variance of nonjunctional current, obtained with 0-mV junctional potential, was subtracted before fitting. This averaged 6% of the fully coupled junctional values and had minimal effect on the analysis.

Estimates of unitary conductance, channel number, and open probability for the noise analysis experiments were derived from the relationships:

$$\sigma^2 = Ii - I^2/N, \quad [1]$$

and

$$I = Npi, \quad [2]$$

where  $I$  is the mean current,  $\sigma^2$  is the variance of the current,  $i$  is the current through a single channel,  $N$  is the number of functional channels, and  $p$  is the open probability (26). In the single channel experiments, unitary conductance was calculated as the change in junctional current divided by the junctional voltage. Unless otherwise stated, all figures are given as the mean  $\pm$  SD.

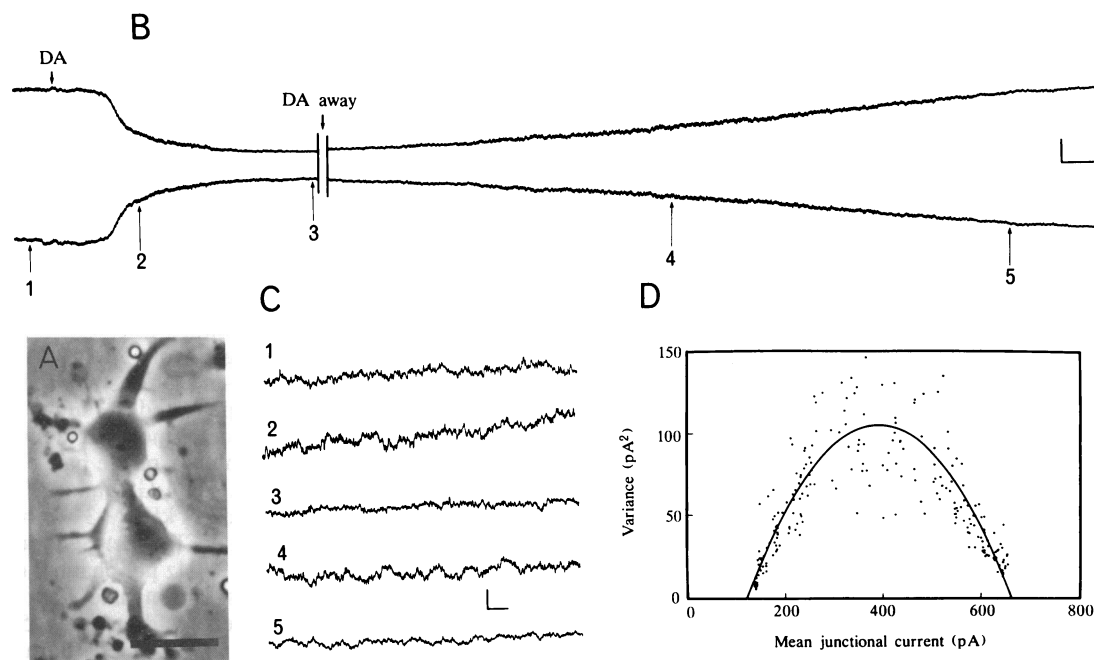
**Solutions.** For most experiments, electrodes were filled with a solution containing 72 mM potassium gluconate, 48 mM KF, 4 mM KCl, 11 mM EGTA, 1 mM CaCl<sub>2</sub>, 1 mM MgATP, 10 mM Hepes (adjusted to pH 7.5 with KOH) (21), and the cells were bathed in a perch Ringer's solution consisting of 145 mM NaCl, 2.5 mM KCl, 20 mM NaHCO<sub>3</sub>, 0.7 mM CaCl<sub>2</sub>, 1 mM MgSO<sub>4</sub>, 20 mM glucose, equilibrated with 97% O<sub>2</sub>/3% CO<sub>2</sub> to give a pH of 7.6 (28). Where noted, solutions designed to block extrajunctional conductances (modified from ref. 27) were used. These consisted of a pipette solution with 120 mM CsCl substituted for the gluconate and fluoride and a bathing solution with 145 mM choline chloride substituted for sodium, plus 20 mM tetra-

ethylammonium and 10 mM 4-aminopyridine. Dopamine (200  $\mu$ M) and octanol (500  $\mu$ M) were freshly dissolved in the bathing solution and ejected onto cells from small-bore pipettes (21, 24).

## RESULTS

Cultures of dissociated white perch retinas contain significant numbers of contacting pairs of horizontal cells. As was found previously (21), most pairs of contacting cells of the same morphological subtype were strongly coupled electrically. Fig. 1A is a photomicrograph of a pair of H1-type horizontal cells in primary cell culture. The activity of gap junction channels between cell pairs was revealed by clamping each of the cells to a different potential, thereby imposing a constant transjunctional voltage gradient. Under such conditions, changes in junctional conductance are reflected as equal and opposite changes in the holding current of the two clamp circuits.

Fig. 1B illustrates the effect of exogenous dopamine on horizontal cell coupling. Dopamine had no consistent effect on the non-junctional resistance of horizontal cells (21, 24) but induced dramatic uncoupling of the cells by decreasing their junctional conductance. In the figure, each trace represents the holding current from one cell of a pair. After a latency of  $\approx$ 10 sec, dopamine (200  $\mu$ M) induced a marked increase in the junctional resistance, reflected as equal and opposite reductions of  $\approx$ 730 pA in the holding current of the two cells. Maximal uncoupling was observed 30–60 sec after the onset of dopamine application, and it could be maintained with repeated dopamine applications (data not shown). Recovery of coupling after removal of the dopamine pipette, shown in the second half of this figure, typically proceeded with a slower time course than uncoupling, often taking several minutes. In the present study, gap junctional con-



**FIG. 1.** Effects of dopamine (DA) on coupling and current noise. (A) Photomicrograph of a contacting pair of H1-type white perch horizontal cells in primary cell culture. (Bar = 30  $\mu$ m.) (B) Dopamine-induced uncoupling and recovery. One cell of the pair was clamped at  $-30$  mV (upper trace), and the other was clamped at  $+10$  mV (lower trace), so that the transjunctional potential was 40 mV (calibrations, 500 pA, 8 sec). (C) Dopamine-induced changes in current noise. One-second segments from the lower trace of the cell pair in B on an expanded time scale (calibrations, 50 pA, 100 msec). Arrows in B indicate approximate locations of segments. (D) Variance analysis of changes in current noise in a similar uncoupling experiment ( $V_j = 40$  mV). Mean and variance of the holding current was calculated for 1-sec intervals, and control values for 0 junctional potential were subtracted to obtain junctional current mean and variance. Line is the least-squares parabolic fit of the data, which gives estimates of 26.7 pS for unitary conductance, 689 for channel number, and a change in open probability from 0.83 to 0.18. Correlation coefficient, 0.83.

ductance was not monitored directly. However, since the change in the input resistance of each cell during uncoupling by dopamine is due solely to changes in junctional resistance, the relative increase in junctional resistance could be inferred. Dopamine induced a 12-fold increase on average in the junctional resistance of 10 well-coupled pairs of cells used for the noise analysis described below, a result consistent with earlier studies (21).

**Noise Analysis.** Changes in the current noise induced by dopamine in one cell of the pair in Fig. 1*B* are shown in Fig. 1*C*. Each trace is an expanded view of the holding current at the times indicated by the arrows in Fig. 1*B*. In the initial highly coupled state (trace 1), junctional conductance was large and current noise was low. As uncoupling began, there was a marked increase in the noise of the holding current, which peaked when the cells were half uncoupled (trace 2). As junctional conductance continued to fall, so did current noise, reaching a value comparable to its predopamine level when the cells were maximally uncoupled (trace 3). A similar pattern of noise changes was observed during recovery of coupling (traces 4 and 5).

We quantified the dopamine-induced changes in current noise by calculating the holding current mean and variance during uncoupling experiments on 10 pairs of cells. If dopamine simply alters the open probability of the gap junction channels, the variance of the current will be related parabolically to the mean current (26). Maximum variance will be observed at open probabilities near 0.5, where at any one moment half the channels are open and half are closed, and variance minima are observed as open probability approaches 1 or 0, where nearly all of the channels are either open or closed. A sample experiment is shown in Fig. 1*D*, which plots the changes in the mean and variance of the junctional current in one cell of a pair during recovery from dopamine. In the uncoupled state, both the junctional current and its variance were small; as coupling increases, the variance increases to a peak and then falls back to a minimum again in the coupled state, where junctional current is largest.

To obtain estimates of channel number, conductance, and open probability, the data from 10 cell pairs were fit with parabolas by the least-squares method. The mean correlation coefficient of the fits was 0.78, indicating an excellent correspondence of the data to the parabolic relationship predicted if dopamine changed open probability. The unitary conductance of junctional channels calculated using Eq. 1 was 32.0 pS ( $\pm 11.0$ ). Estimates of the number of channels between cells ranged from 76 to 1591 (mean,  $562 \pm 433$ ) and was qualitatively related to the contact area of individual cell pairs. Using these values for channel number and conductance, the open probability in the coupled and uncoupled states was calculated for each pair by Eq. 2. Mean values for the 10 pairs were  $p = 0.75$  in the coupled state and 0.14 after application of dopamine, indicating that dopamine substantially reduces the open probability of horizontal cell gap junction channels.

**Unitary Events.** In addition to the estimates obtained by noise analysis, we also measured the amplitude of unitary junctional gating events directly. Such events were observed under conditions of extremely poor coupling as equal and opposite current pulses in the two clamp circuits. A sample recording showing a series of 50- to 60-pS junctional events is in Fig. 2*A*; junctional events from one cell of this pair are shown at higher resolution in Fig. 2*B*. In this cell pair, junctional conductance increased from baseline in  $\approx 50$ -pS steps. Fig. 2*C* is an amplitude histogram of selected events from this cell pair. Most events were in the 50-pS range with second and third peaks in the histogram evident at  $\approx 100$  and  $\approx 150$  pS. A gaussian fit of the first peak gave an average conductance of 51.8 pS, which we take to be the unitary conductance of gap junction channels in this cell pair.

Unitary events were recorded in 11 cell pairs in which the standard pipette solution and Ringer's were used. Four of these pairs were uncoupled by the application of dopamine (200  $\mu$ M), four pairs were uncoupled by the application of octanol (500  $\mu$ M), a universal uncoupler of gap junctions (29), and three pairs had sufficiently poor initial coupling for unitary events to be detected without further treatment. A total of 2346 events were examined. The observed unitary conductance was similar in all three groups; dopamine-treated cells had a single channel conductance of 48.7 pS ( $\pm 6.6$ ), octanol-treated cells had a conductance of 61.1 pS ( $\pm 11.1$ ), and untreated cells had a conductance of 53.2 pS ( $\pm 7.2$ ). These small differences in unitary conductance are not statistically significant ( $P > 0.10$ ; *t* test). Neither dopamine nor octanol thus appears to affect unitary conductance. Averaged together, these cells had a unitary junctional channel conductance of 54.4 pS ( $\pm 9.6$ ).

Unitary gap junctional events were also recorded with a pipette solution with Cs<sup>+</sup> substituted for most of the K<sup>+</sup> and a bathing solution designed to block extrajunctional channels (see *Methods*). Under these conditions, contamination of the records with the activity of extrajunctional channels was reduced, but initial coupling was usually lower than with the standard recording solutions, and recovery of coupling after dopamine application was sometimes slowed. A sample recording and amplitude histogram are shown in Fig. 3. These solutions were used to analyze a total of 2627 events from seven pairs of cells. The unitary conductance of gap junction

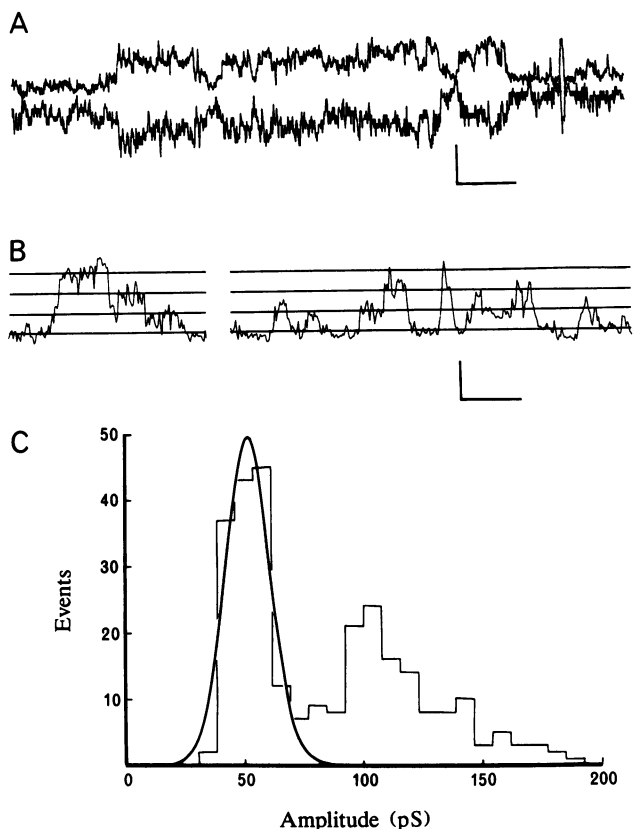


Fig. 2. Single-channel junctional events. (A) Activity of gap junction channels recorded as equal and opposite gating in a poorly coupled cell pair ( $V_j = 50$  mV; calibrations, 5 pA = 100 pS, 250 msec). Openings are represented as the traces moving apart. Events are  $\approx 50$  pS. (B) Selected events from the cell in the upper trace of A at an expanded time scale (calibrations, 5 pA = 100 pS, 100 msec). Horizontal lines show 50-pS levels of conductance. Channel openings are upward. (C) Amplitude histogram of 304 recorded events from the upper cell in A. Gaussian fit of first peak gives a unitary conductance of 51.8 pS ( $\pm 9.2$ ).

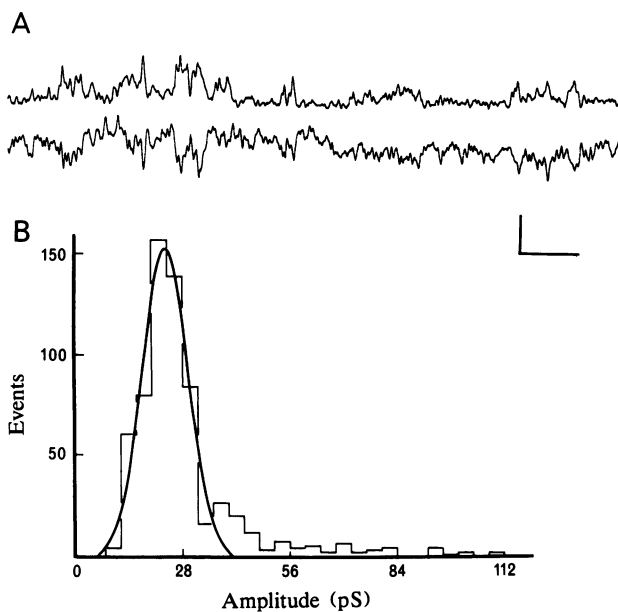


FIG. 3. Single-channel junctional events recorded with  $\text{Cs}^+$  pipette. (A) Lower trace has extrajunctional events (uncorrelated) as well as junctional ( $V_j = 71$  mV; calibrations,  $5$  pA =  $70$  pS,  $50$  msec). (B) Amplitude histogram of 645 junctional events recorded from the cell in the upper trace of A; unitary conductance =  $23.9$  pS ( $\pm 6.1$ ).

channels in dopamine-treated cells ( $30.4 \pm 5.8$  pS;  $n = 4$ ) was indistinguishable from untreated cells ( $31.7 \pm 6.8$  pS;  $n = 3$ ). However, the overall unitary conductance recorded in cells with the  $\text{Cs}^+$ -containing pipette solution ( $30.9 \pm 5.7$  pS;  $n = 7$ ) was significantly reduced compared to the conductance recorded with pipettes containing the normal solution ( $54.4 \pm 9.6$ ;  $P < 0.01$ ;  $t$  test). This suggests that horizontal cell gap junction channels are less permeable to  $\text{Cs}^+$  than they are to  $\text{K}^+$ .

**Modulation of Gating.** Finally, we wished to determine how the dopamine-induced reduction in open probability, shown by noise analysis, is reflected in the gating behavior of individual junctional channels. One way for dopamine to reduce the overall open probability of junctional channels is to reduce their time spent in the open state. To assess this possibility, the duration of junctional events recorded at the

maximum dopamine effect was compared with the duration of events recorded just prior to, or following, maximum uncoupling. One such recording is shown in Fig. 4A. The trace shows the holding current from one cell of a pair. After application of dopamine the cells uncoupled, as indicated by the reduction in holding current. The brackets indicate the sections used for analysis; on the left is the partially uncoupled state, and on the right is the maximally uncoupled state. Inset shows samples of channel activity from partial (upper trace) and maximal (lower trace) sections. Fig. 4B shows that the open duration of junctional events was reduced in the maximally uncoupled state. This was the case in seven cell pairs in which this type of comparison was possible (three from the normal solution group, four from the blocking group). The observed duration of junctional events in the maximally uncoupled state was about half that in the partially uncoupled state (means,  $7.5 \pm 2.3$  vs.  $15.2 \pm 6.0$  msec; 3912 and 3607 events). Since these records contain multiple channels, characterization of the dwell time in the closed state was uncertain.

## DISCUSSION

We conclude from this study that gap junctional channels in teleost horizontal cells have a unitary conductance of 50–60 pS and that dopamine modulates the efficacy of horizontal cell electrical synapses by reducing the open probability of junctional channels. We obtained estimates of unitary conductance both by noise analysis and by direct measurement of junctional gating events. The unitary conductance value obtained by noise analysis was lower than that determined by direct measurement—i.e., 32.0 pS vs. 54.4 pS. However, our variance analysis probably underestimated junctional channel unitary conductance because this method of noise analysis assumes a uniform amplitude for the elementary events giving rise to the recorded current. Violation of this assumption leads to an underestimation of the overall variance, a deviation of the parabola from the origin (see Fig. 1D), and an underestimation of the size of the elementary events (30). While the great majority of current in our experiments was transjunctional current, contamination of the records with the activity of extrajunctional channels did occur to some degree and would decrease the unitary conductance estimate.

The 50- to 60-pS single-channel conductance obtained by direct measurement is similar to that reported for junctional

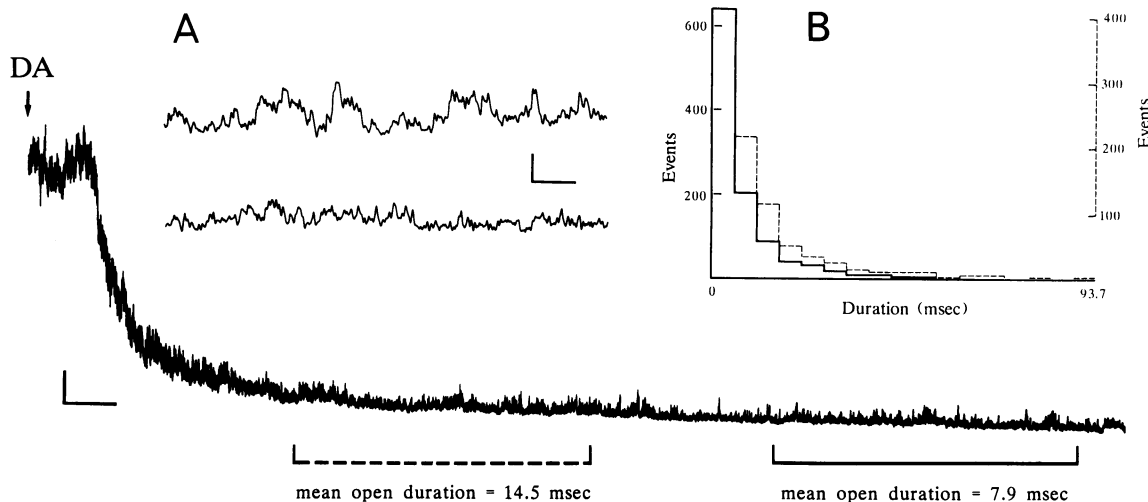


FIG. 4. Dopamine (DA) effects on single-channel events. (A) Dopamine-induced changes in the holding current of one cell of a pair recorded with a  $\text{Cs}^+$ -filled pipette ( $V_j = 60$  mV; calibrations,  $12$  pA,  $6.25$  sec). Brackets show sections used for comparison of partial (dashed) and maximal (solid) dopamine effects. (Inset) Samples of channel activity from partial (upper trace) and maximal (lower trace) sections (calibrations,  $4$  pA =  $67$  pS,  $40$  msec). (B) Open-time histograms of junctional channel openings at partial (dashed) and maximal (solid) dopamine effects. Between the partial and maximal dopamine effect the mean open duration was reduced from  $14.5$  to  $7.9$  msec (925 and 1036 events).

channels in neonatal rat heart cells (13), but it is substantially lower than the estimates of 120–200 pS for channel conductance obtained in other tissues (9–12). All these latter studies also reported smaller junctional events. In our records from poorly coupled cells, the 50- to 60-pS events predominated, and the amplitude of the larger events was generally a multiple of this smallest level (see Fig. 2 *B* and *C*). We interpret this to mean that this 50- to 60-pS conductance is the unitary conductance of horizontal cell gap junction channels and not a subconductance of a larger unitary conductance. Whereas the majority of our observations of junctional channel behavior are consistent with the independent all-or-none gating of 50- to 60-pS channels, there are also instances of slow transitions between conductance levels and concerted gating to higher conductance levels (data not shown). Thus, there may be complexities in channel behavior that remain to be explored.

Our results indicate further that dopamine affects junctional permeability by altering the gating properties of junctional channels, rather than by changing their number or elementary conductance. Noise analysis demonstrated that dopamine uncouples horizontal cells by inducing a dramatic reduction in the open probability of the gap junction channels. In contrast, the unitary conductance of events recorded in cell pairs uncoupled with dopamine or octanol was indistinguishable from untreated cell pairs. In addition, the parabolic relation of variance vs. mean current found in the noise analysis experiments suggests that the number of functional channels was essentially stable during uncoupling with dopamine, at least for the short ( $\leq 5$  min) treatments used here.

The multichannel nature of our recordings imposes some limitations on the analysis of unitary junctional events. While the records clearly indicate the effects of dopamine on open time in a qualitative manner, their usefulness in quantifying these changes is more limited. When cells were maximally coupled with dopamine, the recorded events were almost exclusively unitary so our measurements of open duration under these conditions (7.5 msec) are probably a reasonable approximation of the actual dwell time of individual channels in the open state. In the partially uncoupled state (although most events were still unitary), there are more multilevel openings, which would tend to reduce the apparent open duration (15.2 msec) compared with the actual open time of individual channels (31). Both of these estimates were obtained over a limited range of coupling, near maximum uncoupling, in which unitary events could be resolved and analyzed, so that the change in open duration over the entire range of coupling is presumably much greater. If the entire change in junctional conductance ( $>10$ -fold) is mediated through changes in channel open time, the open duration of junctional channels in the coupled state would be  $\geq 100$  msec, on the order of estimates of junctional channel open time in cardiac myocytes (12, 13). In addition, it is also possible that dopamine affects the closed duration of junctional channels (i.e., their frequency of opening), but this is not easily assessed in our multichannel preparations. Since each hemichannel of a gap junction channel is thought to have its own gate (29), it will be of interest to determine whether the individual hemichannel gates, and dopamine's effects on them, are independent.

The precise relationship between the reduction in junctional channel open time and the biochemical changes induced by dopamine in horizontal cells remains to be elucidated. It is known that dopamine modulates the electrical synapses between teleost retinal horizontal cells through cyclic AMP-dependent protein phosphorylation (22). One attractive hypothesis is that dopamine promotes phosphorylation of the junctional channel proteins themselves. Gap

junction channel proteins purified from liver and heart are substrates for cyclic AMP-dependent protein phosphorylation (6, 32), and application of dopamine to the white perch retina or stimulation of cyclic AMP-dependent phosphorylation in retinal homogenates induces phosphorylation of a membrane protein with a molecular weight similar to known gap junction proteins (33). Whether this is the horizontal cell gap junction channel protein or some other protein involved in synaptic modulation remains to be determined.

We thank Prof. M. V. L. Bennett for helpful comments on the manuscript and Patricia Sheppard for preparation of the figures. This work was supported by National Institutes of Health Grants EY-05886 (D.G.M.) and EY-00824 (J.E.D.) and by a fellowship from The Medical Foundation (A.G.K.).

- Bennett, M. V. L., Verselis, V., White, R. L. & Spray, D. C. (1988) in *Gap Junctions*, eds. Hertzberg, E. L. & Johnson, R. G. (Liss, New York), pp. 287–304.
- Spray, D. C., Saez, J. C., Burt, J. M., Watanabe, T., Reid, L. M., Hertzberg, E. L. & Bennett, M. V. L. (1988) in *Gap Junctions*, eds. Hertzberg, E. L. & Johnson, R. G. (Liss, New York), pp. 227–244.
- Teranishi, T., Negishi, K. & Kato, S. (1983) *Nature (London)* **301**, 243–246.
- Piccolino, M., Gerschenfeld, H. M. & Neyton, J. (1984) *J. Neurosci.* **4**, 2477–2488.
- Lasater, E. M. & Dowling, J. E. (1985) in *Gap Junctions*, eds. Bennett, M. V. L. & Spray, D. C. (Cold Spring Harbor Lab., Cold Spring Harbor, NY), pp. 393–404.
- Saez, J. C., Spray, D. C., Nairn, A. C., Hertzberg, E. L., Greengard, P. & Bennett, M. V. L. (1986) *Proc. Natl. Acad. Sci. USA* **83**, 2473–2477.
- Burt, J. M. & Spray, D. C. (1988) *Am. J. Physiol.* **254**, H1206–H1210.
- Cole, W. C. & Garfield, R. E. (1986) *Am. J. Physiol.* **251**, C411–C420.
- Spray, D. C., Saez, J. C., Brosius, D., Bennett, M. V. L. & Hertzberg, E. L. (1986) *Proc. Natl. Acad. Sci. USA* **83**, 5494–5497.
- Young, J. D.-E., Cohn, Z. A. & Gilula, N. B. (1987) *Cell* **48**, 733–743.
- Neyton, J. & Trautmann, A. (1985) *Nature (London)* **317**, 331–335.
- Veenstra, R. & DeHann, R. L. (1986) *Science* **233**, 872–874.
- Burt, J. M. & Spray, D. C. (1988) *Proc. Natl. Acad. Sci. USA* **85**, 3431–3434.
- Werblin, F. S. & Dowling, J. E. (1969) *J. Neurophysiol.* **32**, 339–355.
- Kaneko, A. (1971) *J. Physiol. (London)* **213**, 95–105.
- Negishi, K. & Drujan, B. (1978) *Sens. Process.* **2**, 388–395.
- Mangel, S. C. & Dowling, J. E. (1985) *Science* **229**, 1107–1109.
- Tornqvist, K., Yang, X.-L. & Dowling, J. E. (1988) *J. Neurosci.* **8**, 2279–2288.
- Van Buskirk, R. & Dowling, J. E. (1981) *Proc. Natl. Acad. Sci. USA* **78**, 7825–7829.
- O'Connor, P., Kropf, R. B. & Dowling, J. E. (1989) *J. Neurochem.* **52**, in press.
- Lasater, E. M. & Dowling, J. E. (1985) *Proc. Natl. Acad. Sci. USA* **82**, 3025–3029.
- Lasater, E. M. (1987) *Proc. Natl. Acad. Sci. USA* **84**, 7319–7323.
- Dowling, J. E., Pak, M. W. & Lasater, E. M. (1985) *Brain Res.* **360**, 331–338.
- Knapp, A. G. & Dowling, J. E. (1987) *Nature (London)* **325**, 437–439.
- Hamill, O., Marty, A., Neher, E., Sakmann, B. & Sigworth, F. J. (1981) *Pflügers Arch.* **391**, 85–100.
- Sigworth, F. J. (1980) *J. Physiol. (London)* **307**, 97–129.
- Lasater, E. M. (1986) *J. Neurophysiol.* **55**, 499–513.
- Yang, X.-L., Tornqvist, K. & Dowling, J. E. (1988) *J. Neurosci.* **8**, 2259–2268.
- Spray, D. C., White, R. L., Campos De Carvalho, A. C., Harris, A. L. & Bennett, M. V. L. (1984) *Biophys. J.* **45**, 219–230.
- Clamann, H. P., Mathis, J. & Luscher, H.-R. (1989) *J. Neurophysiol.* **61**, 403–416.
- Fenwick, E. M., Marty, A. & Neher, E. (1982) *J. Physiol. (London)* **331**, 599–635.
- Pressler, M. L. & Hathaway, D. R. (1987) *Circulation* **76**, Suppl. 18 (abstr.).
- McMahon, D. G. & Dowling, J. E. (1987) *Soc. Neurosci. Abstr.* **13**, 24.