The Journal of Neuroscience, October 1991, 11(10): 3289–3295

Exposure to High-pH Medium Increases the Incidence and Extent of Dye Coupling between Rat Hippocampal CA1 Pyramidal Neurons *in vitro*

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Previous studies have demonstrated that dye coupling between neurons can be reduced by maneuvers that reduce intracellular pH. However, it is apparent that marked extracellular alkaline- as well as acid-going pH shifts can occur in the mammalian CNS. In light of the fact that an applied change in extracellular pH may produce a damped change in intracellular pH in the same direction, in this study we have examined the effects of exposure to high extracellular pH (achieved by raising [HCO_3^-] at a constant P_{CO_2}) on the incidence of Lucifer yellow dye coupling between CA1 pyramidal neurons in the rat hippocampal slice. Under standard conditions (pH 7.4), 44% of CA1 pyramidal neurons were dye-coupled, and the mean number of neurons stained per injection was 1.62. A marked increase in the incidence (88%) and extent (mean number of neurons stained per injection, 3.25) of dye coupling was observed during exposure to high-pH medium (pH 7.9). Under both standard and high-pH conditions, dye coupling was associated with the ability of CA1 pyramidal neurons to generate bursts of action potentials in response to intracellularly applied depolarizing current pulses. The results provide additional evidence that dye coupling between hippocampal pyramidal neurons may be modulated dynamically and may have implications for the genesis of synchronized epileptiform activity under alkalotic conditions.

Changes in extracellular pH (pH_o) can elicit profound effects on cerebral function. For example, raising pH_o can provoke seizures, whereas anticonvulsant activity is associated with extracellular acidosis (Meyer et al., 1961; Aram and Lodge, 1987). Recent experiments in hippocampal pyramidal neurons have shown that perfusion with medium of low pH reversibly attenuates burst firing behavior and depresses evoked field potentials, whereas perfusion with high-pH medium has opposite actions (Balestrino and Somjen, 1988; Church and McLennan, 1989). Nevertheless, while these effects of changes in pH_o may play important roles in the initiation and modulation of synchronous epileptiform activity (see Prince and Connors, 1986), other factors may be involved. In particular, much interest has centered on the possibility that electrotonic coupling between neurons may play a role in synchronizing the neuronal population, in addition to interactions mediated by chemical synapses.

Electrotonic coupling has been studied extensively in the nervous system of nonmammalian preparations (Loewenstein, 1981), and more recently evidence for electrotonic coupling between mammalian central neurons has been presented (reviewed by Dudek et al., 1986). First, gap junctions, which are believed to be the morphological substrates for electrotonic coupling, are widely distributed within the mammalian CNS (e.g., MacVicar and Dudek, 1982). Second, dye coupling between neurons following the intracellular injection of low-molecular-weight fluorescent dyes such as Lucifer yellow (LY), which is believed to be indicative of electrotonic coupling, has been demonstrated *in vivo* (MacVicar et al., 1982) and *in vitro* (e.g., Andrew et al., 1982; MacVicar and Dudek, 1982; O'Beirne et al., 1987). Third, direct electrophysiological demonstrations of coupling have been obtained from simultaneous intracellular recordings of pairs of neurons (e.g., MacVicar and Dudek, 1982; O'Beirne et al., 1987).

Gap junctional conductance is modulated in particular by intracellular [H^+] (Turin and Warner, 1978; Loewenstein, 1981; Spray and Bennett, 1985). Given the proposed importance of electrotonic interactions for the synchronization of neuronal populations during epileptogenesis, together with the fact that extracellular alkalosis is associated with the development of epileptiform activity, in this study we have examined the possibility that exposure to high-pH medium might increase the extent of dye coupling between hippocampal CA1 pyramidal neurons. Furthermore, it was recently reported that CA1 pyramidal neurons that display bursts of action potentials in response to depolarizing current pulses under pH_o 7.4 conditions are nearly always dye-coupled to other pyramidal cells, whereas those neurons that respond to depolarizing current pulses by generating a train of repetitive spikes are not (Baimbridge et al., 1991). With the knowledge that exposure to high-pH media promotes the appearance of intrinsic burst discharges (Church and McLennan, 1989), we have in addition examined whether the apparent correlation between dye coupling and burst firing behavior seen under normal (i.e., pH_o 7.4) conditions is maintained during exposure to high-pH medium.

Some of these data have been presented previously in an abstract (Church et al., 1989).

Materials and Methods

Transverse hippocampal slices (400 μm) were prepared from adult Wistar rats as previously described (Church and McLennan, 1989). They

Received Feb. 20, 1991; revised Apr. 25, 1991; accepted May 20, 1991.

We wish to thank Ms. Stella Atmadja for her expert technical assistance. This work was supported by the Medical Research Council of Canada (J.C.) and the British Columbia Health Research Foundation (K.G.B.). J.C. is the recipient of a Scholarship from the British Columbia Health Research Foundation.

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were placed on a nylon mesh in a low-volume (<0.5 ml) recording chamber at the interface between an oxygen-enriched (95% O_2, 5% CO_2), humidified atmosphere and artificial cerebrospinal fluid (aCSF) at a temperature of 34.5 ± 0.2°C. Slices were allowed to recover for 60 min before recordings were made.

The standard perfusion medium contained (in mM) NaCl, 129.5; KCl, 5; $NaHCO_3$, 21; NaH_2PO_4, 1.25; $MgSO_4$, 2; $CaCl_2$, 2; and D-glucose, 10. It was continuously gassed with 95% O_2/5% CO_2, giving a pH of 7.4, and was perfused at a rate of 1.5 ml/min. High-pH test aCSF contained 61 mM $NaHCO_3$ (pH 7.9 after equilibration with 95% O_2/5% CO_2); the change in $NaHCO_3$ concentration was balanced by an equimolar change in NaCl. The pH of the aCSF was measured continuously with a glass electrode placed in a small reservoir either immediately up- or downstream from the recording chamber.

Intracellular recordings were obtained from the somata of CA1 pyramidal neurons with microelectrodes pulled from thin-walled capillary tubing (1.0 mm o.d., 0.75 mm mm i.d.) and filled with either 1 M K-acetate or 5% Lucifer yellow CH (Sigma Chemical Co.) in distilled, deionized water. The LY solution was freshly prepared and filtered (0.22 μm) prior to use. Final resistances were 40–75 MΩ (K-acetate–filled) and 90–210 MΩ (LY-filled). Microelectrodes were connected to an active bridge circuit (Axoclamp 2, Axon Industries Inc.) that allowed the simultaneous injection of current and measurement of the membrane potential. The indifferent bath electrode was a 3 M KCl, 4% agar bridge.

In each experiment, slice viability was established by impaling a CA1 pyramidal neuron with a K-acetate–filled microelectrode during perfusion with standard aCSF (pH 7.4) and observing characteristic responses to direct depolarization and orthodromic synaptic stimulation. LY-filled microelectrodes were then used, impalements being carried out during perfusion with standard aCSF (pH 7.4), at various intervals after the start of perfusion with high-pH (7.9) aCSF, and at various times after the start of reperfusion with standard aCSF following periods of exposure to high-pH aCSF. An impalement was obtained by advancing an electrode in 2 μm steps by means of a Burleigh piezoelectric microdrive; ringing the negative capacitance feedback was avoided as this can lead to spurious dye ejection (see Rao et al., 1986). Immediately after the successful impalement of a neuron, its resting membrane potential (V_m) was recorded, and its active firing properties were characterized by injecting a series of depolarizing current pulses from V_m. This procedure never took more than 120 sec. In control experiments, electrophysiological characterizations were performed in the above fashion with LY-filled microelectrodes, which were then rapidly withdrawn; no detectable LY staining was subsequently observed. In some neurons, membrane input resistance (R_{in}) was estimated by injecting a series of current pulses (>100 msec duration) from V_m and measuring the resulting hyperpolarizing electrotonic potentials. Only neurons with V_m > −55 mV that displayed a train of overshooting action potentials in response to a square-wave depolarizing current pulse were injected with LY. Poor-quality impalements obtained while searching for a stable penetration were terminated in <60 sec without active LY injection; in no case was detectable staining noted under these conditions.

Intracellular recordings made with LY-filled microelectrodes are generally of poorer quality than those made with non-LY-containing solutions, in part because of high electrode resistances. In addition, the intracellular injection of Li^+ ions blocks the delayed rectifier K^+ channel (Mayer et al., 1984), an effect that might contribute to burst generation (see below; Schwartzkroin and Prince, 1980). Nevertheless, the fact that, under standard conditions, both regular-spiking and bursting responses to depolarizing current pulses could be obtained with LY-filled microelectrodes and that the distinction between them could be made before any broadening of the action potential occurred, indicates that the possible effect of Li^+ ions on the type of response to depolarizing current pulses under our experimental conditions was likely to be small.

LY was injected by applying 350 msec hyperpolarizing current pulses (2–3 nA, 2 Hz) for periods of between 3 and 6 min. The injection was terminated and the electrode withdrawn if the membrane potential suddenly declined, if the neuron became less excitable (i.e., did not generate a train of action potentials in response to a depolarizing current pulse), or after 6 min of filling, whichever came first. A maximum of two well-separated dye injections were performed in any individual slice, and only one dye injection was attempted in a single electrode track. Once impaled, no attempt was made to improve the penetration by movement of the electrode (see Knowles et al., 1982). Following LY injection, slices were immersion-fixed overnight at 4°C in 10% formalin containing 1% calcium acetate. The fixed slices were washed in saline and then left for 4 hr in saline containing 20% sucrose. They were then mounted onto a previously cut frozen block of embedding medium (O.C.T. compound, Canlab) and rapidly frozen with Cryoquik (Canlab). Sections from 22 to 30 μm were collected into 0.1 M phosphate-buffered saline and scanned under low power on a Carl Zeiss Axiophot microscope equipped for epifluorescence [fluorescein isothiocyanate (FITC) filters] in order to locate LY-filled neurons. Dye coupling was assessed blind regarding the conditions under which the neuron was filled.

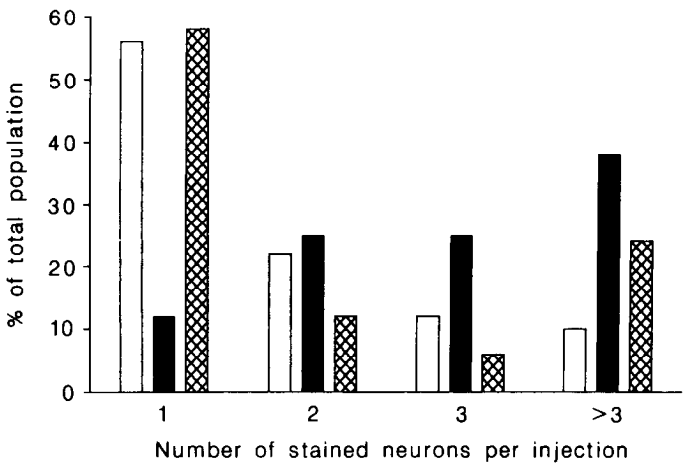

Figure 1. Frequency histogram of the number of stained neurons per successful dye injection under standard (pH 7.4) conditions (*open bars*), during perfusion with high-pH (pH 7.9) aCSF (*solid bars*), and during reperfusion with standard medium after exposure to high-pH aCSF (*cross-hatched bars*).

The electrophysiological data were photographed directly from the screen of an analog oscilloscope (Tektronix 5113) or were displayed on a chart recorder (Gould 2200S). While the chart recorder traces accurately reflect the amplitude of slower potential changes, the frequency response of the instrument is insufficient to follow fast action potential discharges, and these appear truncated in figures. Some data were stored on magnetic tape (Racal Store 4DS; bandwidth, 0–2.5 kHz) for later analysis.

A χ^2 test was used to compare the incidence of dye coupling between the experimental groups, and a *t* test was employed to assess the significance of the difference in the mean number of neurons stained per injection under the various experimental conditions.

Results

Only those neurons in which electrophysiological responses could be reliably classified into regular-spiking and bursting types (see below) and in which staining with LY was subsequently found to be intense enough to enable the clear evaluation of the presence or absence of dye coupling were accepted for analysis. Staining was judged acceptable when the apical and basal dendritic trees, as well as the soma, were clearly visible. A total of 93 neurons fulfilled these criteria. Of the total, 42 injections were made in standard aCSF (21 mM HCO_3^-, pH 7.4), 34 in high-pH aCSF (pH 7.9), and 17 in standard aCSF after exposure to high-pH aCSF for various periods of time.

Data obtained under standard (pH 7.4) conditions

Under standard conditions, 44% of neurons injected with LY were dye-coupled. This incidence is similar to that previously reported by other investigators in the neocortex and various hippocampal subfields of the rat (Andrew et al., 1982; Connors et al., 1983, 1984; MacVicar and Jahnsen, 1985). Also in agreement with previous studies, where dye coupling was observed it was usually to only one other pyramidal neuron. Thus, the

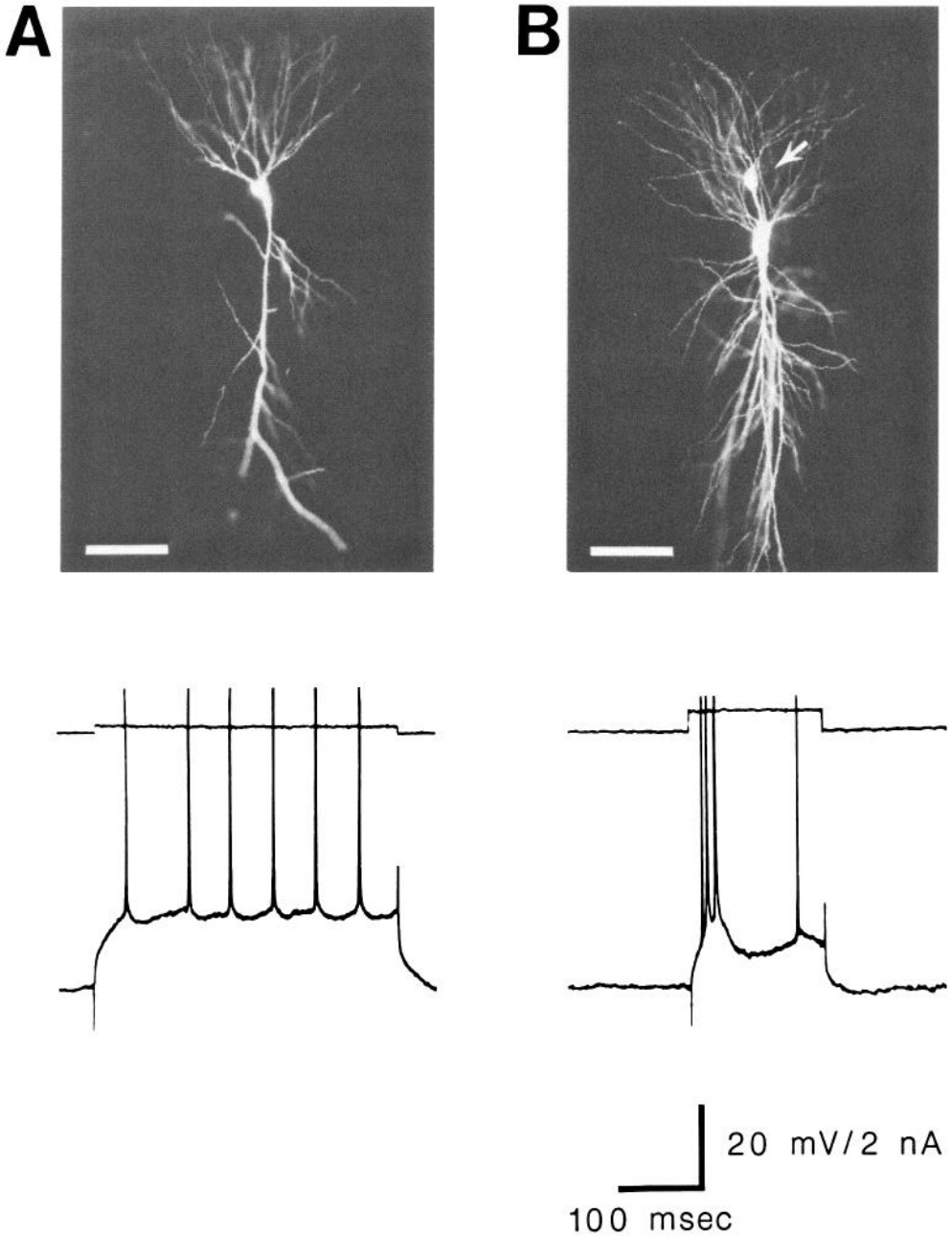

Figure 2. LY-stained hippocampal CA1 pyramidal neurons injected during perfusion with standard (pH 7.4) aCSF. *A,* Single, noncoupled, pyramidal neuron. *B,* An aggregate of three neurons. In *B* note the pattern of two neurons in the superficial layer of the stratum pyramidale coupled to a single neuron lying below the deep layer (*arrow*). Scale bars, 100 μm. The electrophysiological responses of the neurons in *A* and *B,* evoked by depolarizing current pulses from V_m (−62 mV and −64 mV, respectively), are shown *beneath* the photomicrographs and were classified as regular-spiking and bursting, respectively. *Upper traces,* electrode current; *lower traces,* membrane potential. Action potential amplitudes are truncated in this and subsequent figures for convenience of figure preparation.

mean number of pyramidal cells (±SEM) stained per injection was 1.62 ± 0.14, a value similar to that reported by Rao et al. (1987) following the injection of 5,6-carboxyfluorescein into a mixed sample of CA1 and CA3 pyramidal neurons *in vitro* (1.56 ± 0.18). A frequency histogram of the number of stained neurons per successful dye injection is presented in Figure 1. Examples of a single and a (rare) multiple fill obtained following a single injection of LY under standard conditions are shown in Figure 2. On five out of nine occasions when three or more neurons were found in a dye-coupled aggregate, it was noted that the aggregate consisted of one or more neurons close together in the superficial layer of the stratum pyramidale (i.e., at the stratum pyramidale/stratum radiatum border; Lorente de Nó, 1934) coupled to a single neuron lying below the deep layer (i.e., below the stratum pyramidale/stratum oriens border; Fig. 2). A similar pattern of dye coupling has been illustrated, but not commented upon, by others (e.g., MacVicar et al., 1982, their Fig. 1). The clear separation argues strongly against artifactual dye coupling and may have functional consequences (see Discussion).

Rat hippocampal CA1 pyramidal neurons under standard (i.e., pH 7.4) conditions respond to depolarizing current pulses in two distinctive forms. "Regular-spiking" neurons exhibit a repetitive series of Na^+-dependent action potentials, whereas less common "bursting" neurons display an initial burst of fast spikes superimposed upon an underlying depolarization (Masukawa et al., 1982; Church and McLennan, 1989; see also McCormick et al., 1985). Examples of the two types of response, recorded with K-acetate–filled microelectrodes, are shown in Figure 3, *A* and *B*. As with recordings obtained with K-acetate–filled microelectrodes, the most common response to depolarizing current pulses observed with LY-filled electrodes under

standard conditions consisted of a repetitive sequence of fast spikes (i.e., regular-spiking cells). Examples of regular-spiking and bursting responses recorded with LY-filled microelectrodes during perfusion with standard aCSF are shown in Figure 2. Of the total sample of 42 neurons, 25 were classified as regular-spiking and, in all but three cases, were not dye-coupled. In two of the three exceptions the injected neuron was dye-coupled to one other neuron, and in the remaining case to two other neurons. Conversely, bursting neurons were, in all 17 cases, found to be dye-coupled. In agreement with previous studies (e.g., Church and McLennan, 1989; Bilkey and Schwartzkroin, 1990; Chagnac-Amitai et al., 1990), there was little difference in V_m or R_{in} between regular-spiking and bursting neurons, and thus between noncoupled and coupled neurons. V_m values for regular-spiking, noncoupled neurons and bursting, coupled neurons were 66 ± 8 mV (mean ± SD) and 64 ± 10 mV, respectively, whereas R_{in} values were 28 ± 9 MΩ (n = 8) and 24 ± 8 MΩ (n = 8), respectively. Note that these values were obtained within 120 sec of the impalement of a neuron (see Mayer et al., 1984; Tseng and Haberly, 1989).

Effects of perfusion with high-pH medium

A marked increase in the incidence of dye coupling occurred during perfusion with high-pH aCSF for periods of between 4 and 115 min. A χ^2 test revealed that the difference in the incidence of coupling under high-pH conditions (88%) and the control incidence (44%) was highly significant (χ^2 = 25.65; df = 1; $p < 0.001$). Not only was the incidence of dye coupling greater under high-pH conditions, but also the number of dye-coupled cells per aggregate was increased. Injected cells were often coupled to two or more (occasionally to as many as eight) other neurons (Figs. 1, 4). The mean number of cells (±SEM) stained per injection was 3.25 ± 0.22, compared to 1.62 ± 0.14 under standard conditions. Thus, perfusion with high-pH aCSF produced a large and statistically significant ($p < 0.001$, t test) increase in the number of neurons in a dye-coupled aggregate. The increase in the extent of dye coupling was noted within 10 min of the start of perfusion with high-pH aCSF and did not appear to develop further with continued exposure.

Perfusion with high-pH medium is often associated with the conversion of a regular-spiking pattern of action potential discharge to a bursting pattern (Church and McLennan, 1989). An example of such a conversion, obtained with a K-acetate–filled microelectrode, is shown in Figure 3. Of the 34 neurons recorded with LY-filled microelectrodes during perfusion with high-pH aCSF, 32 (94%) were classified as bursting, and all but two were found to be dye-coupled. Of the two neurons classified as regular-spiking under high-pH conditions, neither were dye-coupled. Examples of LY-filled neurons injected under high-pH conditions, together with their electrophysiological responses, are shown in Figure 4. Perfusion with high-pH aCSF produced little change in V_m values compared with those obtained under standard conditions, in agreement with previous data (Church and McLennan, 1989), nor did perfusion with high-pH medium alter the observation made under standard (pH 7.4) conditions of a lack of difference in values for V_m between regular-spiking, noncoupled and bursting, coupled neurons. Similarly, R_{in} for regular-spiking, noncoupled neurons was 26 ± 8 MΩ (mean ± SD; n = 2), little different from the value of 27 ± 10 MΩ (n = 13) found in bursting, coupled neurons.

Seventeen injections of LY were made in hippocampal slices that had been exposed to high-pH aCSF for periods of between 20 and 115 min and then reperfused with standard (pH 7.4) aCSF. The frequency histogram of the number of stained neurons per successful dye injection (Fig. 1) was similar to that obtained following LY injections into neurons that had never been exposed to high-pH conditions. The mean number of cells (±SEM) stained per injection in this group, the injections being made within 10–60 min of the start of reperfusion with control aCSF, was 1.78 ± 0.27. This value is slightly higher than that in the control group (1.62 ± 0.14), reflecting the residue of neurons coupled to three or more others seen in the frequency histogram, but the difference did not reach statistical significance ($p > 0.20$, t test).

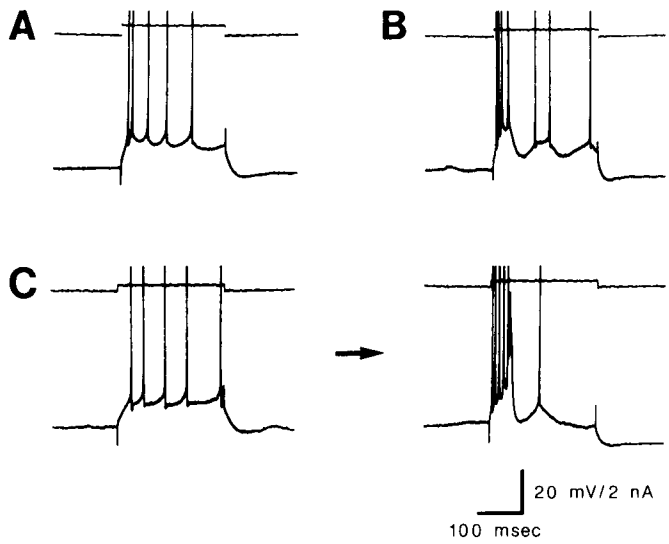

Figure 3. Electrophysiological responses of three different hippocampal CA1 pyramidal neurons to depolarizing current pulses recorded with K-acetate–filled microelectrodes. *Upper traces*, electrode current; *lower traces*, membrane potential. Responses in *A*, *B*, and the *left-hand trace* in *C* were obtained during perfusion with pH 7.4 aCSF and were classified as regular-spiking, bursting, and regular-spiking, respectively. The *right-hand trace* in *C* was obtained 20 min after the start of perfusion with pH 7.9 aCSF; this maneuver resulted in the conversion of the regular-spiking response seen under pH 7.4 conditions to a bursting response (see Church and McLennan, 1989). All responses were obtained from the following values of V_m: *A*, −62 mV; *B*, −60 mV; *C*, −65 mV.

Discussion

Raising the pH of the external medium by increasing [HCO_3^-] at a constant P_{CO_2} increases the incidence and extent of dye coupling between rat hippocampal CA1 pyramidal neurons *in vitro*. Alkalinization of the external medium will elicit a change in intracellular pH (pH_i) in the same direction, albeit slower and smaller (see Roos and Boron, 1981), and this would appear to be the likely mechanism by which the increase in dye coupling is mediated, given that gap junctional conductance is sensitive to changes in pH_i (Turin and Warner, 1978; Spray and Bennett, 1985). Although data concerning a change in pH_i consequent upon a change in pH_o in a HCO_3^-/CO_2-buffered system are not available for hippocampal CA1 pyramidal neurons, Tolkovsky and Richards (1987) reported that raising pH_o from 7.4 to 7.8 in the presence of HCO_3^- caused pH_i in cultured rat superior cervical ganglion (SCG) neurons to rise from 7.19 to about 7.31. Quantitative data concerning the relationship between pH_i and gap junctional conductance are also unavailable for hippocampal pyramidal cells, but on the assumption that this relationship

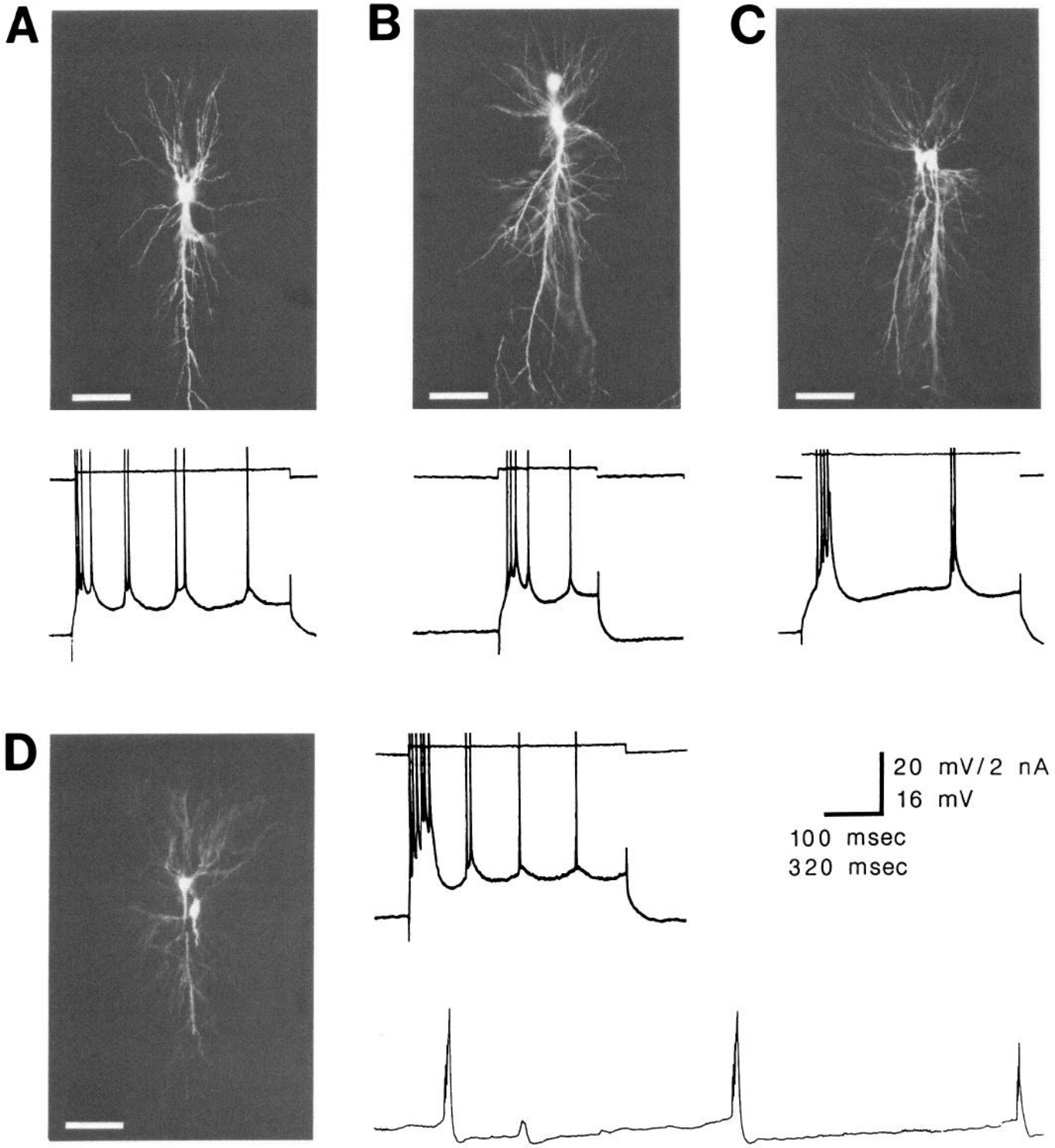

Figure 4. Hippocampal CA1 pyramidal neurons filled with LY during perfusion with high-pH aCSF. *A–C* show examples of dye-coupled aggregates of two, three, and four neurons, respectively. Injections were made 20, 33, and 30 min after the start of perfusion with pH 7.9 aCSF, respectively. Electrophysiological responses to depolarizing current pulses are shown *beneath A–C*. These were obtained from V_m (−60 mV, −65 mV, and −63 mV, respectively) and were all classified as bursting. *Upper traces,* electrode current; *lower traces,* membrane potential. Two neurons from a dye-coupled aggregate of three neurons (one neuron was located in an adjacent section) are shown in *D*, the LY injection being made 35 min after the start of perfusion with pH 7.9 aCSF. The burst firing response to a depolarizing current pulse from V_m (−66 mV) is shown to the *right* (*upper trace,* electrode current; *lower trace,* membrane potential), together with a chart record of spontaneous epileptiform activity recorded at V_m, just prior to injecting LY. Note different calibration (16 mV, 320 msec) for chart record. Scale bars, 100 μm.

is relatively steep, as it is in many other electrically coupled biological systems (see Spray and Bennett, 1985), a rise in pH_i in hippocampal CA1 pyramidal neurons of the magnitude known to occur in SCG neurons following a change in pH_o similar to that employed in the present experiments might be expected to produce a marked rise in gap junctional conductance.

Not only does perfusion with high-pH medium lead to overt epileptiform discharges *in vitro* (Aram and Lodge, 1987), but hyperventilation, which raises CSF pH (Merwarth and Sieker, 1961), has long been used as a diagnostic test for subclinical seizure disorders and prolongs seizure duration in humans undergoing electroconvulsive therapy (e.g., Chater and Simpson, 1988). Alkalosis affects a wide variety of factors that may be of importance for the development of seizure discharges in the mammalian CNS (see Balestrino and Somjen, 1988; Church and McLennan, 1989). To this list must now be added an increase in the extent of dye and, by implication, electrotonic coupling, given the suggested contribution of electrotonic interactions to synchronization of neuronal populations during epileptogenesis. Furthermore, bursting neurons have been implicated in the initiation of epileptiform discharges (Connors, 1984), and the combination of an increase in both burst firing activity and electrotonic coupling evoked concomitantly by high-pH conditions might be expected to be potently epileptogenic.

Burst firing can be elicited in electrotonically coupled molluscan neurons by a maintained or short (10 msec) depolarizing stimulus delivered to a single neuron in the coupled aggregate, which then exhibits a burst of action potentials as a consequence of regenerative excitation through electrotonic synapses between the coupled neurons (Getting and Willows, 1974). It is an intriguing possibility that a similar process, which for its initiation depends only on the presence of nonrectifying electrotonic synapses of a type that has been demonstrated between central mammalian neurons (e.g., MacVicar and Dudek, 1982; O'Beirne et al., 1987), may play a role in burst generation in electrotonically coupled hippocampal CA1 pyramidal neurons. In this case, a neuron within an electronically coupled aggregate that is initially depolarized might be further depolarized by regenerative excitatory feedback, into the range of membrane potential where activation of various subthreshold nonactivating or slowly inactivating Na^+ and Ca^{2+} currents would occur (Johnston et al., 1980; MacVicar, 1985). These currents would add an active depolarizing component to the membrane depolarization produced by the junctional currents. Finally, higher-threshold Na^+ and Ca^{2+} currents would be activated and result in burst generation. Electrotonic interactions, which can act on a rapid (sub-millisecond) time scale (see Dudek et al., 1986), might in this fashion promote burst firing in CA1 pyramidal neurons whose behavior in response to a depolarizing current pulse would otherwise be restricted to simple repetitive firing.

Even if dye coupling and burst firing are not causally associated, the fact that they are nearly always correlated and that their frequency of occurrence can be experimentally manipulated in a reversible fashion lends support to the proposition that both parameters may be determined by common extra- or intracellular modulators. Of particular interest with regard to the present experiments and the control of both dye coupling and burst firing are changes in $[H^+]$. Thus, maneuvers that would be expected to lower pH_i, such as exposure to weak acids or high concentrations of CO_2, have previously been shown to reduce the incidence of dye coupling (Gutnick and Lobel-Yaakov, 1983; Connors et al., 1984; MacVicar and Jahnsen, 1985; O'Beirne et al., 1987), whereas an increase in pH_i is the likely basis for the increase in dye coupling seen in the present experiments. Changes in pH_o, and thus also pH_i, are also able to elicit marked effects on the pattern of action potential discharge in response to depolarizing current pulses in hippocampal CA1 pyramidal neurons. In particular, perfusion with low-pH medium attenuates burst firing, whereas exposure to high-pH aCSF the same as that employed in the present experiments leads to the development of burst discharges (Church and McLennan, 1989).

Certain anatomical features may also contribute to the development of burst discharges in a dye-coupled aggregate. Of particular note is our present observation that dye-coupled aggregates frequently consist of a pair of neurons located at the stratum pyramidale/stratum radiatum border (i.e., the superficial layer of stratum pyramidale; Lorente de Nó, 1934) coupled to a single neuron lying below the stratum pyramidale/stratum oriens border (i.e., below the deep layer of the stratum pyramidale). It is noteworthy that the single neuron in these aggregates has a large primary apical dendrite, a feature that has been associated with burst firing pyramidal neurons in both the hippocampal CA3 region (Bilkey and Schwartzkroin, 1990) and neocortex (Chagnac-Amitai et al., 1990).

It is well established that reductions in pH_o can occur during normal synaptic transmission, as well as pathophysiological events such as ischemia/hypoxia and seizure activity, but more recently it has become apparent that extracellular alkaline transients can also occur in the mammalian CNS (see Chesler, 1990). Given that changes in pH_o may evoke changes in pH_i in the same direction (see above), the possibility exists that the increase in dye coupling observed in the present experiments may be seen *in vivo* under circumstances of a sustained rise in tissue pH. For example, cortical pH values tend to be more alkaline in neonatal than in adult rats, a finding that may reflect greater $[HCO_3^-]_i$ because it occurs in the face of elevated tissue levels of CO_2 (Woodbury et al., 1984; Johanson et al., 1988). The present results may therefore be relevant to the findings of Connors et al. (1983) and Christie et al. (1989) that the incidence of dye coupling between neonatal rat cortical and locus coeruleus neurons, respectively, is higher than seen in the adult and that the number of neurons per dye-coupled aggregate also decreases with age.

In conclusion, we have found that exposure to high-pH medium increases the incidence and extent of dye coupling between hippocampal CA1 pyramidal neurons *in vitro*. That the increase in dye coupling is associated with an increase in burst firing activity serves to reinforce the fact that changes in pH can exert a marked effect on neuronal function, even if the two parameters are not causally related.

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