Mechanism of Transmitter Release: Voltage Hypothesis and Calcium Hypothesis

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The calcium hypothesis of synaptic transmission has been challenged by experimental results using the crayfish neuromuscular junction that suggest that presynaptic depolarization can trigger transmitter release directly without calcium influx. Results from electrophysiological experiments using the same preparation do not support this voltage hypothesis, but are consistent with the calcium hypothesis. Voltage may modulate, but not elicit, transmitter release.

S YNAPTIC TRANSMISSION CONSISTS OF THE PRESYNAPTIC secretion of a transmitter and a postsynaptic response. As in other forms of secretion, calcium is believed to play a crucial role. When an action potential invades a presynaptic nerve terminal, the depolarization opens calcium channels in the presynaptic membrane. The influx of calcium leads to a transient rise in the intracellular calcium concentration adjacent to the plasma membrane. These calcium ions trigger directly, by an unknown mechanism, a transient release of neurotransmitter called phasic release for 1 or 2 msec. This account of the process of transmitter release is called the calcium hypothesis of transmitter release (1).

Related to the calcium hypothesis of transmitter release is a proposed mechanism of synaptic facilitation, the residual calcium hypothesis (2–5). Residual calcium activity following phasic release is thought to cause an increase in tonic release, manifested as an increased frequency of spontaneously released quanta of transmitter in the absence of electrical or presynaptic stimulation (recorded as miniature excitatory postsynaptic potentials or MEPSP's). In addition, this residual calcium is thought to enhance phasic release of transmitter during subsequent action potentials (synaptic facilitation). These effects last nearly 1 second.

Substantial evidence indicates that calcium influx is needed for evoked transmitter release. Calcium ions must be present in the external medium during the action potential (δ), suggesting that their entry into the terminal is triggered by depolarization. If synaptic terminals are focally depolarized by an intense current, the excitatory postsynaptic potential (EPSP) is delayed until the end of the depolarization (7). This "off-EPSP" suggests that depolarization which exceeds the calcium equilibrium potential retards calcium entry. At the squid giant synapse there is a close correlation between transmitter release and calcium influx measured directly with voltage clamp (8). Other evidence supports a role for calcium in neurosecretion. Increasing the intracellular calcium whether by presynaptic calcium injection (9) or by exposure to calcium-transporting ionophores (10), metabolic poisons (11), or with calcium-filled liposomes (12) elicits transmitter release. Action potentials can reduce the rate of transmitter release when the electrochemical calcium gradient across the membrane is reversed, as in calcium-free medium (13).

An alternative model for the mechanism of transmitter release has been postulated (14-19). Presynaptic depolarization resulting from an action potential is thought not only to admit calcium ions, but also to act independently and directly on the presynaptic secretory machinery to release transmitter. In this way presynaptic depolarization could trigger release of transmitter by a direct effect, without any influx of calcium ions. Although calcium ions are still needed for the process of release, residual calcium from prior activity or the resting level of intracellular calcium may provide sufficient calcium ions if the depolarization is strong enough. We refer to this model as the voltage hypothesis of transmitter release (Fig. 1).

This alternative model is based on the following observations: (i) A brief train of action potentials facilitates subsequent transmitter release evoked by a small depolarizing pulse 50- to 1600-fold (14, 15). The calcium and residual calcium hypotheses cannot account for such dramatic facilitation in the absence of a marked increase in MEPSP frequency. It was suggested that the test pulse elicits release in the virtual absence of calcium influx, by a direct action of depolarization in the presence of residual calcium from prior activity. (ii) As the amplitude of the first of a pair of focal depolarizations (14, 15, 17) is increased, it evokes more transmitter release and also facilitates release in response to the second pulse. Very large first pulses evoke less facilitation, presumably because calcium influx is reduced as the calcium equilibrium potential is approached. Nevertheless, release increases in response to a large first pulse, suggesting a direct effect of depolarization. (iii) The nearly constant time course of transmitter release evoked by depolarizing pulses of varying amplitude (16, 18, 20) suggests that something other than calcium (perhaps presynaptic potential) determines the time course of release; different amounts of intracellular calcium might be expected to show different time courses under these circumstances. (iv) Large depolarizations approaching the calcium equilibrium potential at the squid giant synapse release more transmitter than small depolarizations admitting equal amounts of calcium (8), suggesting a direct effect of potential on release. $\left(v\right)$ Theoretical analysis (19) of the calcium hypothesis (21) produces quantitative predictions about the calcium-dependence of the magnitude of release and time course that are not observed experimentally (4, 20), suggesting that some other factor (presynaptic potential) controls release.

New tests of the voltage hypothesis. Considerable evidence thus exists in support of both calcium and voltage hypotheses of trans-

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Fig. 1. Two hypotheses of mechanisms underlying transmitter release. In the calcium hypothesis (left), depolarization simply opens calcium channels. The resulting calcium influx causes a rise in intracellular calcium at release sites ("active calcium") which triggers release. The alternative voltage hypothesis (right) suggests a direct effect of depolarization on release sites which activate them by an unspecified mechanism. The activated sites may react with preexisting internal calcium to release transmitter.

mitter release. Using the same crayfish neuromuscular junction preparation as Dudel, Parnas, and Parnas (14, 18), we designed experiments to dissociate the effects of calcium influx and presynaptic potential.

Hyperosmotic medium increases tonic transmitter release (that is, spontaneous MEPSP frequency in the absence of stimulation) (22). Apparently this occurs because of increased internal sodium concentration (23), which is then thought to elevate presynaptic calcium (24). The voltage hypothesis (Fig. 1) predicts that presynaptic depolarization will enhance transmitter release in a calcium-free medium (where calcium influx is impossible), if internal calcium is elevated by hyperosmotic treatment. We found that depolarization with high potassium did not elevate transmitter release (25); on the

contrary, it reduced release rate (Fig. 2). We also found that this reduction in release is blocked by 13.5 mM cobalt (a calcium channel blocker). These results suggest that, when the electrochemical gradient for calcium is reversed by elevating internal and reducing external calcium, depolarization simply opens calcium channels and efflux of calcium reduces internal calcium and therefore release.

Carbonyl cyanide *m*-chlorophenylhydrazone (CCCP) was used to uncouple mitochondrial transport and to elevate presynaptic calcium by another mechanism. At 10 μ M, CCCP increases spontaneous MEPSP frequency by a factor of 100 (26). We depolarized a fine nerve branch directly with current pulses rather than with potassium. Even after internal calcium was increased by CCCP treatment, depolarization failed to release transmitter in a calcium-free medium (Fig. 3). In some experiments, action potentials also failed to evoke release in calcium-free or cobalt-containing media which included CCCP. These results are consistent with the calcium hypothesis, but not the voltage hypothesis.

Reexamination of evidence for the voltage hypothesis. The results and arguments cited in support of the voltage hypothesis can be interpreted in terms consistent with the calcium hypothesis.

The train-pulse experiment demonstrated that, after a train, a small pulse often released about as much transmitter as an action potential would at that moment. Crustacean peripheral axons have depolarizing afterpotentials (27) which cause a postactivation supernormal excitability in motor neuron terminals (28). Therefore, a subtreshold pulse could sometimes become suprathreshold after a tetanus. Many of these motor neuron terminals are excitable (29, 30), and some excitable release zones would likely be under the opening of the electrode. Therefore, averaged records of posttetanic pulse-evoked responses might include spike-evoked responses. The large facilitation of pulse-evoked release would be caused by a previously subthreshold stimulus sometimes evoking spikes after a train.



Fig. 2. Effect of depolarization of nerve terminals on spontaneous transmitter release in a hyperosmotic calcium-free medium (42). MEPSP frequency drops slightly when calcium is removed from the medium. When osmolarity is increased with sucrose, MEPSP frequency consistently rises (43). Increasing external potassium (from 5.4 mM) depolarizes muscle fibers about 23 mV, as expected for an imperfectly potassium-selective membrane, and presumably does the same to nerve terminals. In this solution transmitter release does not increase, even transiently, but drops instead, apparently due to the efflux of calcium through open calcium channels. Finally, MEPSP frequency returns to initial levels (horizontal dotted line) in normal Ringer. This result was seen in five preparations.



Fig. 3. Lack of evoked transmitter release in calcium-free medium after mitochondrial uncoupling. In each panel, the top trace shows postsynaptic membrane potential at a fast sweep speed. Phasic release appears as a positive wave (EPSP) when the terminals are depolarized by a 2-msec pulse. The bottom trace shows spontaneous release of miniature EPSP's recorded postsynaptically at a slower sweep speed. (A) Depolarizing pulses evoked release in normal Ringer containing calcium. (B) Phasic release was entirely absent, and MEPSP frequency slightly reduced, in calcium-free medium containing 5 mM EGTA. (C) CCCP (10 μ m) was added, but depolarizing pulses never evoked phasic release, even when MEPSP frequency increased 100-fold. (D) Phasic release was facilitated and MEPSP frequency was still relatively high, presumably due to residual active calcium from the CCCP treatment (44). These results were seen in five preparations.

We tested this explanation of the train-pulse experiment by comparing posttetanic facilitation in the presence and absence of action potentials. After a train of action potentials, a small pulse may release hundreds of times as many quanta as it does in isolation, with larger pulses showing less facilitation (Fig. 4, A to C) (31). This result is similar to that of Dudel *et al.* (14, 15). When we used tetrodotoxin to block action potentials, and substituted a train of depolarizing pulses adjusted to release as many quanta as spikes had, then the test pulses showed facilitation ratios of only 1.5 to 10 (in different experiments) (Fig. 4, D to H). Evidently, the post-train effect of small pulses was boosted by the triggering of action potentials, or perhaps abortive spikes, in terminals during the postactivation supernormal excitability period.

We also measured the facilitation of a small test pulse following a train at different intervals (Fig. 4I). Facilitation started at high levels shortly after a train of action potentials, and declined gradually until 110 msec, when it dropped suddenly to very low values. Such a curve is not characteristic of the decay of posttetanic facilitation at this synapse (32). Rather, it indicates that at 110 msec, the postactivation supernormal excitability decayed sufficiently that the test pulse no longer elicited an action potential, and so transmission dropped precipitously.

In the two-pulse experiment, increasing pulse amplitude elicits increasing transmitter release in response to that pulse and decreasing release to a subsequent pulse. This result is clarified by an analysis of the spatial distribution of extracellular potential in the opening and under the rim of a macro patch electrode. Huxley and Taylor and Stühmer *et al.* (33) showed that the potential profile at the tip of an electrode similar to that used in our experiments (Fig. 5A) was not uniform. Macro patch electrodes pressed against neuromuscular junctions have a resistance of about 1 megohm (29), only 0.1 percent of the resistance of a 50- μ m patch of membrane. Thus 99.9 percent of the current passes under the rim of the electrode. This leads to a sharp voltage gradient under the rim, with a shallower gradient within the patch itself (33). Since the rim thickness is large and similar to the inside radius, a large number of nerve branches under the rim are exposed to this gradient of stimulus intensity (Fig. 5B).

The effect of increasing pulse intensity is diagrammed in Fig. 5C. The effective depolarization of the nerve membrane, which is roughly proportional to the external field, drops off from the inner edge of the rim to the outer edge of the pipette. If phasic transmitter release occurs for presynaptic potentials between some threshold value (θ) and the calcium equilibrium potential (E_{Ca}), then small pulses elicit release only from nerve terminals under the electrode opening (central dotted area of Fig. 5, C and D). Larger pulses elicit release from a wider area under the rim (central dotted and surrounding shaded areas in Fig. 5, C and D). This recruitment of additional release zones leads to a larger postsynaptic response. Still larger pulses elicit release only from nerve terminal regions under the

Fig. 4. The train-pulse experiment. Traces (A) to (F) are computer-averaged recordings of postsynaptic current measured with a macro patch electrode (45). Transmitter release was estimated by counting the number of quanta released in repeated trials. (A) A 2-msec, 0.45-µA depolarizing test pulse releases an average of one quantum every 34th trial. Thus the average quantal content is 0.029. (B) When the same pulse is delivered 17 msec after a train of six presynaptic action potentials at 83 Hz, it releases much more transmitter (12.6 quanta on average). Facilitation (F) is expressed as the ratio of release by a pulse of a given amplitude following the train to release by the pulse in isolation. Release to the spikes also facilitates. (C) When 2-msec, 0.4-µA depolarizing pulses through the patch electrode are substituted for action potentials, they elicit release similar to that following spikes in (B). Release in response to the test pulse is similar to (B) (13.7 quanta). (D) After addition of tetrodotoxin (TTX) (200 ng/ml) to the bath, the test pulse is about as effective in releasing transmitter as in (A) (average quantal content is 0.009). (E) The train of pulses that mimicked spikes in (B) is ineffective in releasing transmitter or in facilitating release to the test pulse (quantal content equals 0.013). The conditioning pulses in (B) required the triggering of presynaptic action potentials for facilitation of the test pulse response. (F) A train of larger pulses, 0.6 µA in amplitude, now mimics the action potentials of (B) in releasing transmitter. However, the test pulse releases only slightly more transmitter than in (D) (0.046 quanta). Thus TTX blocked the large facilitation of the test pulse response caused by the tetanus. The large facilitation in (B) was apparently caused by the test pulse eliciting a spike when it followed a train, due to the postactivation supernormal excitability known to occur at these motor neuron terminals (28). (G) Transmitter release evoked by a 2-msec test pulse, before and after adding TTX to the bath. (H) Facilitation of release evoked by test pulses when they are preceded by trains of action potentials (83 Hz; 17-msec interval between train



and pulse) without TTX present. Also shown is facilitation of release evoked by test pulses where the train of action potentials is replaced by a train of conditioning pulses in TTX, which released the same amount of transmitter as the action potentials. The posttetanic test pulse shows much less facilitation when spikes are blocked. (G) and (H) are from a different experiment than (A) to (E).

(I) In a third experiment, a train of six action potentials (77 Hz) precedes a 2-msec, 0.3-µA test pulse. Release by the test pulse is plotted as a function of the interval between train and test pulse. The sudden drop in facilitated release at 110 msec suggests that up to this interval, the test pulse elicited an action potential after the train. These results were seen in six preparations.

electrode rim, where the potential lies between θ and E_{Ca} (outer hatched ring of Fig. 5, C and D). Nevertheless, both the area of release and the postsynaptic response are large. Only very large pulses elicit a reduced total release from a narrow zone at the outer edge of the electrode (29).

Consider the situation of a large pulse followed by a small test pulse. The large pulse admits calcium only in the peripheral terminals under the rim, but still elicits a large response. Even 500 msec later, residual calcium will be confined to the peripheral nerve branches since intracellular calcium diffuses very slowly—only about 3 μ m in 0.5 second, or a fraction of the distance from peripheral to central nerve sites. However, the small test pulse admits calcium only in the central zone, where it cannot add to residual calcium that entered at the periphery.

The above considerations predict that a small test pulse will be facilitated by only a small first pulse, while a large test pulse will be facilitated most by a large first pulse. This is what was observed (Fig. 5, E to H). As the size of the first pulse increased, it elicited increasing responses. Small test pulses were facilitated maximally by small first pulses. Medium tests pulses were facilitated maximally by medium first pulses. Large test pulses were facilitated maximally by large first pulses. If the depolarization of the nerve terminals under the electrode were uniform, as suggested previously (16), this result is difficult to explain (31).

The assertion that the calcium hypothesis does not predict a constant time course of release due to the decay of active calcium after different size pulses, or in the presence of differing degrees of facilitation (16, 18, 19), is refuted by recent simulations of the calcium hypothesis. Stockbridge and Moore and Fogelson and Zucker (34) showed that the time course of active calcium is the same, within the precision of experimental measurement techniques, for facilitated and unfacilitated release. This is also true for pulses of different sizes (35).

Recent work by Augustine *et al.* (36) indicates that the apparent voltage-dependence of release from the squid giant synapse observed by Llinás *et al.* (8) and Smith *et al.* (37) is not seen under all experimental conditions. Moreover, simulations of calcium diffusion away from individual presynaptic channels indicate that as large



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Fig. 5. The two-pulse experiment. (A) Photomicrograph of the macro patch electrode. Outer diameter is 100 μ m. (B) Approximate current lines for stimulation through the macro patch electrode. The electrode is pressed lightly against a muscle fiber and neuromuscular junctions. (C) Spatial potential profiles under macro patch electrode $(\hat{3}3)$. A weak pulse depolarizes only a small central region of the patch above the threshold for transmitter release ($\hat{\theta}$). Calcium influx and transmitter release occur only in this central zone [central dotted area in (D)]. A larger pulse activates most of the patch under the opening of the electrode [dotted and shaded areas in (D)]. A very large pulse activates only release zones under the rim of the electrode, because the central region is depolarized to a level which is greater than the calcium equilibrium potential (E_{Ca}) . Calcium influx and transmitter release occur only in the outer hatched area in (D). (E) The original two-pulse experiment consists of a variable first pulse (P1) followed by a small test pulse (P2). We perform three separate experiments, using small, medium, and large test pulses [solid, dotted, and dashed lines, respectively in (E) to (H)]. (F) Average number of quanta released by P1 in all three experiments. (G) Average number of quanta released by P2 in all three experiments. (H) Facilitation of release by test pulse in all three experiments (ratio of release by P2 with and without P1). Panels (F) to (H) are averages of 120 responses. The standard errors are about 20 percent for responses above 0.2 quanta in (F) and 10 percent for most points in (\vec{G}) . The macro patch technology is the same as in Fig. 4 (45).

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depolarizations open more channels, there is greater overlap of calcium diffusing to release sites from neighboring channels, resulting in elevated active calcium concentrations and higher release rates (38). This does not occur at lower potentials which open fewer, widely separated channels. This can account for the apparent voltage-dependence of release (8, 37) under the calcium hypothesis, without postulating an actual voltage-dependence of release.

Theoretical objections to the calcium hypothesis have also been made. Parnas and Segel (19) show that a special formulation of the calcium hypothesis predicts that the time course of release will depend on prior activity and external calcium, and that release will be a nonsaturating function of presynaptic calcium activity. They cite evidence that release time course is invariant (4, 20) and that release is a saturating function of presynaptic calcium activity (21) as arguments against the calcium hypothesis.

There are problems with this analysis: (i) A first-order removal process is used which is appropriate only for uniform presynaptic calcium in a well-stirred terminal. Simulations of presynaptic calcium diffusion (34, 35) indicate that active calcium does not decline according to a first or higher order one-compartment removal equation. (ii) The temperature-dependence of the synaptic delay (39) indicates that processes subsequent to the decay of active calcium become rate-limiting at low temperature. Release time course should not track that of active calcium accurately, and may not resemble it at all. (iii) Most important, that release is a saturating function of active calcium was concluded from the finding that facilitation measured at brief intervals is reduced in elevated extracellular calcium (21, 40). However, early facilitation may only appear reduced because of a superimposed depression due to depletion of transmitter stores which is more pronounced at high calcium levels. Such a calcium-sensitive depression concurrent with facilitation is well known at many neuromuscular junctions [see, for example, (41)], and appears to be present at the junctions used in these studies (32). This depression could account for the effect of calcium on facilitation. Moreover, experimental evidence (32) indicates that release does not show saturation at the low levels of calcium proposed (19, 21) by the theoretical work.

Further aspects of release. We find no evidence that transmitter release is evoked by depolarization in the absence of calcium influx, whether depolarization is elicited by action potentials, short electrical pulses, or exposure to high potassium solutions; and the result is the same even if presynaptic calcium is elevated pharmacologically. We find that the evidence supporting the voltage hypothesis can be explained by the calcium hypothesis, when certain procedural artifacts are considered. We therefore conclude that the calcium hypothesis is the most parsimonious explanation of a large body of experiments, and provides the most useful basis for further work.

There is the possibility that transmitter release can be modulated by membrane potential (17). Brief pre- or post-pulses appear to alter release to a test pulse, but leave facilitation unchanged when it is measured with an identical test pulse presented later. As long as both test pulses are the same, our spatial model (Fig. 5) indicates that they will initially activate and subsequently test the same terminal regions. Then unchanged facilitation implies unchanged residual calcium and unchanged calcium influx during the first test pulse. The pure calcium hypothesis would then predict no modulatory effect of pre- or post-pulses on release. Such an effect suggests that the presynaptic potential immediately before or after a depolarizing pulse can influence release caused by calcium influx during that pulse. However, this does not imply that depolarization can elicit release in the absence of calcium influx, only that it can affect release caused by an influx of calcium ions. This is an aspect of synaptic transmission requiring further investigation.

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- Depolarization with high potassium might reduce MEPSP amplitude to below the noise level due to the reduction in postsynaptic driving force and increased input conductance. Then an increase in transmitter release in calcium-free medium might be missed. However, control experiments in normal calcium medium showed that we could easily detect the increase in MEPSP frequency, and that the amplitude distribution of MEPSP's was only slightly affected by depolarization with high otassium.
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 The effect of pulse amplitude on location of excited terminals (Fig. 5) is relevant to Fig. 4H. Without tetrodotoxin, a small pulse weakly activates central synapses. After a conditioning train, this test pulse triggers a spike, eliciting widespread release and a large facilitation. A large pulse already elicits higher release levels from a large number of peripheral terminals. This response is not subject to as great an increase by a spike triggered by the test pulse after the train, and will therefore show less facilitation. Similarly, Fig. 5H shows that in tetrodotoxin, a small pulse is more subject to facilitation by a train of medium size pulses than is a large pulse. 31. more subject to facilitation by a train of medium size pulses than is a large pulse. Facilitation to a large pulse is reduced for two reasons. It activates different terminals than the conditioning pulses, and the initial response is due to a
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 All experiments in this article were performed on the neuromuscular junction of the claw oppner muscle of the first two walking less of the crayfish Procambarus

- All experiments in this article were performed on the neuromuscular junction of the claw opener muscle of the first two walking legs of the crayfish *Procambarus darkii*. Legs from animals about 8 cm long were used. The dissection, mounting, and illumination of the preparation, superfusion of media, motor neuron stimula-tion, and intracellular recording of MEPSP's from the opener muscle have all been described previously [J. Dudel and S. W. Kuffler, J. Physiol. (London) 155, 514 (1961)]. The normal Ringer solution was van Harreveld's crayfish Ringer with 10 mM HEPES to buffer pH to 7.3 (5). Calcium-free medium contained an additional 13.5 mM magnesium, plus 5 mM EGTA to chelate extracellular calcium, and the pH was adjusted to 7.2. Hypercentic solution contained 200 mM sucrose Potasium was adjusted to 7.3. Hyperosmotic solution contained 200 mM sucrose. Potassium was substituted for sodium in high potassium medium. Intracellular electrodes were 1 to 5 megohms and filled with 4M potassium acetate. Temperature was 22° to
- 43. The delay in rise in MEPSP frequency probably represents the time required for terminal shrinkage and elevation of intracellular calcium. The transient fall in MEPSP frequency upon adding sucrose is not always seen.
- In Fig. 4, a 20-µm (outer diameter) suction electrode was used to depolarize a fine nerve branch by the electrotonic method (30). Intracellular recordings were made from a muscle fiber adjacent to the suction electrode. The records shown in the

upper traces are averages of 40 responses. The stimulus artifact was reduced by subtracting from each record a standard baseline trace consisting of the average of ten responses of quantal failures (traces with no transmitter release). Action potentials were blocked with 200 ng/ml tetrodotoxin. CCCP was dissolved at 1 mM in dimethyl sulfoxide (DMSO), which was added to the bathing medium to give a final concentration of 10 μ M CCCP and 0.1 percent DMSO. Temperature vas 13°C

45. In Fig. 4, transmitter release is recorded as postsynaptic current from a large number of release sites in motor neuron terminals lying under a macro patch electrode. The latter is a fire-polished pipette, 50 μm (inner diameter) and 100 μm (outer diameter) connected in a voltage clamp configuration [see 16, 29, and J. Dudel, Pflügers Arch. Gesamte Physiol. Menschen Tiere 391, 35 (1981)]. Stimulating pulses were applied to the presynaptic terminals through the patch pipette, using

the circuits of Dudel (14, 29) to reduce stimulus artifacts. Each trace is the average of 80 responses when pulses follow trains, and 500 responses when pulses are given alone. With this number of trials, standard errors were less than 20 percent for alone. With this number of trials, standard errors were less than 20 percent for most points. Stimulus regimes were repeated at 0.1 Hz. Residual artifacts following the test pulse have been removed by subtracting from each record a baseline trace consisting of the average of ten responses of quantal failures to the test pulse alone. Spikes in Fig. 4B were elicited by stimulating the excitatory nerve with a suction electrode in the meropodite of the leg. Temperature was 13°C. We thank R. English for assistance with the electronics, and him and S. Dao for help with the figures. J. Giovannini provided assistance with some of the experiments. Supported by NIH grant NS 15114.

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Role of Seasonality in the Evolution of Climate During the Last 100 Million Years

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A simple climate model has been used to calculate the effect of past changes in the land-sea distribution on the seasonal cycle of temperatures during the last 100 million years. Modeled summer temperatures decreased over Greenland by more than 10°C and over Antarctica by 5° to 8°C. For the last 80 million years, this thermal response is comparable in magnitude to estimated atmospheric carbon dioxide effects. Analysis of paleontological data provides some support for the proposed hypothesis that large changes due to seasonality may have sometimes resulted in an ice-free state due to high summer temperatures rather than year-round warmth. Such "cool" nonglacials may have prevailed for as much as one-third of the last 100 million years.

URING THE LAST 100 MILLION YEARS THE EARTH'S CLImate has changed from an ice-free state to one with polar ice caps (1). Previous modeling studies (2) have attempted to determine whether changing land-sea distribution may have contributed to temperature changes in high latitudes. However, simulated temperature changes do not seem to be large enough to trigger ice-cap formation. We now discuss a feature that might account for such a large climate change. We propose that changes in seasonality may have played a key role in the formation of polar ice caps. The present seasonal cycle is primarily controlled by the landsea distribution (3). We suggest that past changes in the seasonal cycle were affected by changes in the land-sea distribution-the drift of continents into high latitudes, and the opening of polar seaways. Formulating a hypothesis in terms of seasonality allows us to focus on perhaps the single boundary condition most critical for maintenance of permanent ice cover-the summer temperature. Regardless of how cold it gets in the winter, it is not possible to initiate ice cap formation if it gets too hot in the summer. The hypothesis is based on the geological boundary condition known best about the past (the land-sea distribution) and it is supported by calculations involving a two-dimensional energy balance climate model.

Our modeling approach differs from previous model studies (4) that were mostly conducted with mean annual heating distributions (no seasons) and which stipulated high albedo (snow cover) for land areas in high latitudes. We include the seasons and do not allow snow-albedo changes. This approach isolates the role of seasonality without the complication of a strong and uncertain feedback mechanism. The reason that the analogous experimentation with three-dimensional general circulation models (GCM's) has not been conducted is the large expense incurred in obtaining the equilibrium seasonal cycle (at least 15 model years need to be simulated to approach equilibrium in a passive oceanic mixed layer). Furthermore, present GCM's are unable to reliably simulate precipitation in high latitudes. Restricting analyses to mean annual heating has resulted in the conclusion (4) that reasonable changes in albedo, cloudiness, and oceanic heat transport cannot explain the estimated large thermal changes and that changing atmospheric carbon dioxide levels (5) may have to be invoked in order to account for the differences. Inclusion of the seasonal cycle may significantly alter this conclusion.

Our study is focused on the interval (80 million to 20 million years ago) between the maximum ice-free state of the mid-Cretaceous (6) and the time that is generally accepted as the minimum age for the formation of the Antarctic ice cap (1). The general trend of climate change is summarized in Fig. 1. Although detailed interpretation of this record has been questioned (7), comparison of the data with other paleoclimatic indices (8-10) suggests that, on balance, the Late Cretaceous-early Cenozoic had less ice than the present.

Geologic setting. Plate reconstructions for the last 100 million years indicate the continued breakup of Pangaea, with the net result

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