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Examination of the Role of cGMP in Long-term Potentiation in the CA1 Region of the Hippocampus

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Abstract

The mechanisms underlying the generation of NMDA receptor-dependent LTP in the CA1 region of the hippocampus continue to receive a great deal of attention because of the postulated importance of LTP as a synaptic mechanism for learning and memory. It is well accepted that the initial induction of LTP occurs in the postsynaptic cell, but the site of expression remains controversial. One prominent hypothesis is that LTP involves the release of one or more retrograde messengers that act on the presynaptic terminal to enhance transmitter release. Recently, evidence has been presented that retrograde messengers function to activate presynaptic guanylyl cyclase and that the resulting rise in presynaptic cGMP levels, when accompanied by presynaptic activity, is responsible for generating an early component of LTP. We have tested this hypothesis by examining whether synaptic strength is increased by coupling tetanic stimulation with application of a membrane-permeable analog of cGMP. The experiments were done in the presence of an NMDA receptor antagonist to block postsynaptic induction mechanisms. Under a variety of experimental conditions, this manipulation failed to generate LTP, suggesting that an increase in cGMP levels accompanied by presynaptic activity is not sufficient to generate LTP in the CA1 region of the hippocampus.

Introduction

Long-term potentiation (LTP) in hippocampal CA1 synapses has served as a model for the cellular changes that underlie learning and memory in mammals, and there is, thus, great interest in elucidating its molecular mechanisms. It is now well accepted that the induction of LTP depends on processes in the postsynaptic cell, specifically activation of N-methyl-D-aspartate (NMDA) receptors and the consequent rise in postsynaptic calcium concentration (for review, see Bliss and Collingridge 1993; Nicoll and Malenka 1995). However, the site of expression has been difficult to determine definitively, and, as yet, no consensus has been reached (Kullmann and Siegelbaum 1995). Nevertheless, it is clear that if the expression of LTP involves enhanced release of neurotransmitter, a retrograde messenger, produced in the postsynaptic cell and affecting presynaptic function, is mandatory. Several molecules have been suggested to play such a role, including arachidonic acid, carbon monoxide (CO), and nitric oxide (NO) (Bliss and Collingridge 1993; Williams et al. 1993a; for review, see Bear and Malenka 1994). Because all of these molecules can activate guanylyl cyclase, a particularly attractive

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hypothesis is that activation of this enzyme and the resultant increase in presynaptic cGMP levels coupled with presynaptic activity cause a long-lasting enhancement in transmitter release. Directly supporting this hypothesis is the demonstration that a tetanic stimulus given in the presence of an NMDA receptor antagonist elicits LTP if a membrane-permeable analog of cGMP is present (Zhuo et al. 1994). Furthermore, it was found that in culture, loading presynaptic hippocampal cells with cGMP also caused LTP when paired with a tetanus, again given in the presence of an NMDA receptor antagonist (Arancio et al. 1995). Additional evidence for the involvement of cGMP was the finding that LTP can be blocked by inhibitors of guanylyl cyclase or cGMP-dependent protein kinase (Zhuo et al. 1994; Arancio et al. 1995). The conclusion from these experiments was that LTP involves the generation of a retrograde messenger that activates presynaptic guanylyl cyclase. The resulting increase in presynaptic cGMP levels then causes a sustained enhancement of transmitter release from terminals that were activated while cGMP levels were elevated.

Other investigators have had difficulty in observing the same effects of cGMP analogs on synaptic strength (Irving et al. 1993; Schuman et al. 1994). One possible explanation for this apparent discrepancy is that, like the ability of NO synthase inhibitors to block LTP (Haley et al. 1993; Williams et al. 1993b), the ability of cGMP to cause LTP depends critically on the experimental conditions. Because of the potential significance of these observations in elucidating the site and mechanisms of LTP expression, we have reexamined the effects on synaptic strength of pairing tetanic stimulation (in the presence of an NMDA receptor antagonist) with application of 8-bromo-cGMP using a variety of experimental conditions in several different laboratories. This experiment was deemed critical because it bypasses postsynaptic NMDA receptors during the induction of LTP and strongly implicates processes within the presynaptic cell as being important for the generation of LTP.

Materials and Methods

Standard procedures were used to prepare hippocampal slices from either young rats (14–26 days old) (New York and Boston groups) or adult female guinea pigs (San Francisco group). After at least a 1-hr recovery period, slices were transferred to the recording chamber where they were submerged beneath a continuously superfusing solution saturated with 95% O_2 and 5% CO_2. A cut was made between the CA3 and CA1 regions to prevent propagation of epileptiform activity. The composition of solutions and the conditions varied slightly between laboratories. The Boston group used a solution containing (in mM): NaCl, 120; KCl, 2.5; $MgSO_4$, 1.3; $CaCl_2$, 2.5; NaH_2PO_4, 1; $NaHCO_3$, 26; and dextrose, 10. The temperature was 30°C. Stimulation strength was set to produce 50% maximal response. The New York group used two solutions: one the same as that of Zhuo et al. (1994) (four experiments) and the other the standard one used in the New York group laboratory (five experiments) (in mM): NaCl, 119; KCl, 2.5; $MgCl_2$, 1.3; $CaCl_2$, 2.5; $NaHCO_3$, 26.2; NaH_2PO_4, 1; and D-glucose, 11. Results with the two solutions were similar and thus were pooled. Picrotoxin (100 μM) was used in all experiments. The temperature was 25°C. Stimulation strength was set to produce 50% of the maximal response. The San Francisco group used a solution identical to that used by Zhuo et al. (1994). Picrotoxin (100 μM) was used in all experiments. The temperature was 30°C. The stimulation strength was set so that the amplitude of the field EPSP was 0.5–0.7 mV. Standard extracellular recording techniques were used to record field EPSPs in the stratum radiatum. The New York group stimulated a single pathway at the rate of 0.033 Hz. The Boston group alternately stimulated two independent inputs, each at the rate of 0.033 Hz. The San Francisco group alternately stimulated two independent inputs, each at the rate of 0.02 Hz. Data were acquired and analyzed on-line using custom software. Summary graphs were generated as described previously (Malinow et al. 1988; Huang et al. 1992). All summary data is reported as mean ±S.E.M. Drugs used were 8-bromo-cGMP (RBI, CalBiochem), DL-APV (Sigma, RBI), Picrotoxin (Sigma), and D-APV (Tocris Neuramin).

This collaboration grew out of our common interest in the role of second messengers in LTP. It was agreed to test in parallel a previously reported crucial experiment in which potentiation could be induced by cGMP analogs, despite block of the NMDA channel (Zhuo et al. 1994). It was agreed that each group would use its own methodology. We further agreed that the results would not be shared until data analysis was complete and final summary graphs were constructed.

Results

NEW YORK GROUP EXPERIMENTS

In this set of experiments, the perfusing medium initially contained DL-APV (100 μM) and synaptic transmission was monitored until a stable baseline of 10 min was obtained (<1% change in the EPSPs averaged over the first 5 min compared with the last 5 min, $n = 9$; Fig. 1). Following establishment of this baseline, 100 μM 8-bromo-cGMP was applied to the preparation. As illustrated in Figure 1A, this produced no consistent effects on synaptic strength. After allowing 7.5 min for the drug to perfuse the tissue (control experiments established that 2–3 min is adequate for 10 μM CNQX to completely block the field EPSPs; data not shown), a series of tetanic stimuli were delivered. Each tetanus consisted of 10 stimuli delivered at 100 Hz and nine such tetani were delivered separated by 30 sec. Synaptic strength was monitored beginning 30 sec after the last tetanus and was minimally affected by the tetanic stimulation when measured 20 min after the end of the tetanic stimulation (5±1% increase, average of EPSPs from 16–20 min after the tetani compared with the 5-min average preceding the tetani; Fig. 1A).

To test whether these same slices were capable of generating LTP, the 8-bromo-cGMP and DL-APV were washed out for 20–30 min ($n = 8$ of the nine experiments). After again ensuring that synaptic transmission was stable for at least 10 min, the same series of tetani were delivered that now elicited stable LTP (Fig. 1B). Synaptic strength was increased by 80±1% when measured 15–20 min after the tetani and 51±3% when measured 45–50 min after the tetani.

BOSTON GROUP EXPERIMENTS

In these experiments, the perfusing medium initially contained DL-APV (50 μM) and synaptic strength was monitored until a stable 10- to 15-min baseline was obtained. Following establishment of this baseline, 100 μM 8-bromo-cGMP was applied to the preparation for 5 min at which time a tetanus (100 Hz, 1 sec) was applied to one of the two inputs. This caused a small increase in response (10±2%, $n = 5$; Fig. 2) when measured 30 min after the tetanus. A small increase was also seen in the unstimulated pathways, and so this change is not considered significant. To test whether these same slices were capable of generating LTP, the 8-bromo-cGMP and DL-APV were washed out for 35 min. After again ensuring that synaptic transmission was stable for 10–15 min,
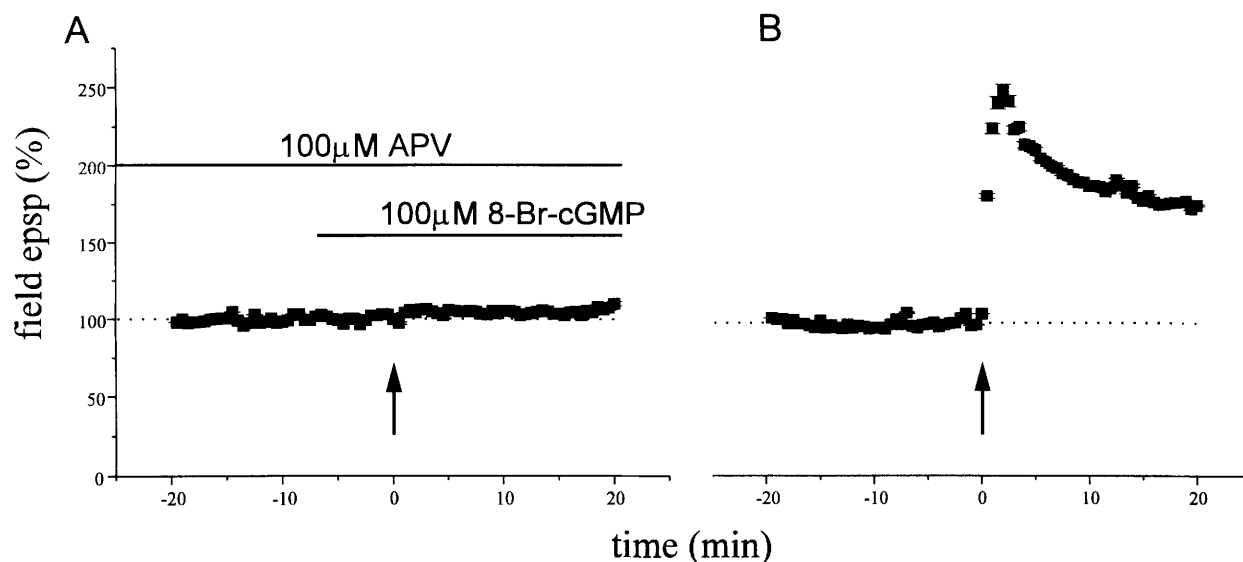

Figure 1: Results from the New York group indicate that the block of LTP by APV is not overcome by 8-bromo-cGMP. (*A*) Ensemble averages from nine experiments monitoring synaptic strength versus time. Slices initially bathed in 100 μM DL-APV. After a stable baseline was established, 100 μM 8-bromo-cGMP was added to the bath; 7.5 min later, a series of tetani were delivered (arrow; see text). (*B*) In eight of the nine slices, cGMP and APV were washed out, a new baseline was established, and the same series of tetani were delivered (arrow). This generated robust LTP.
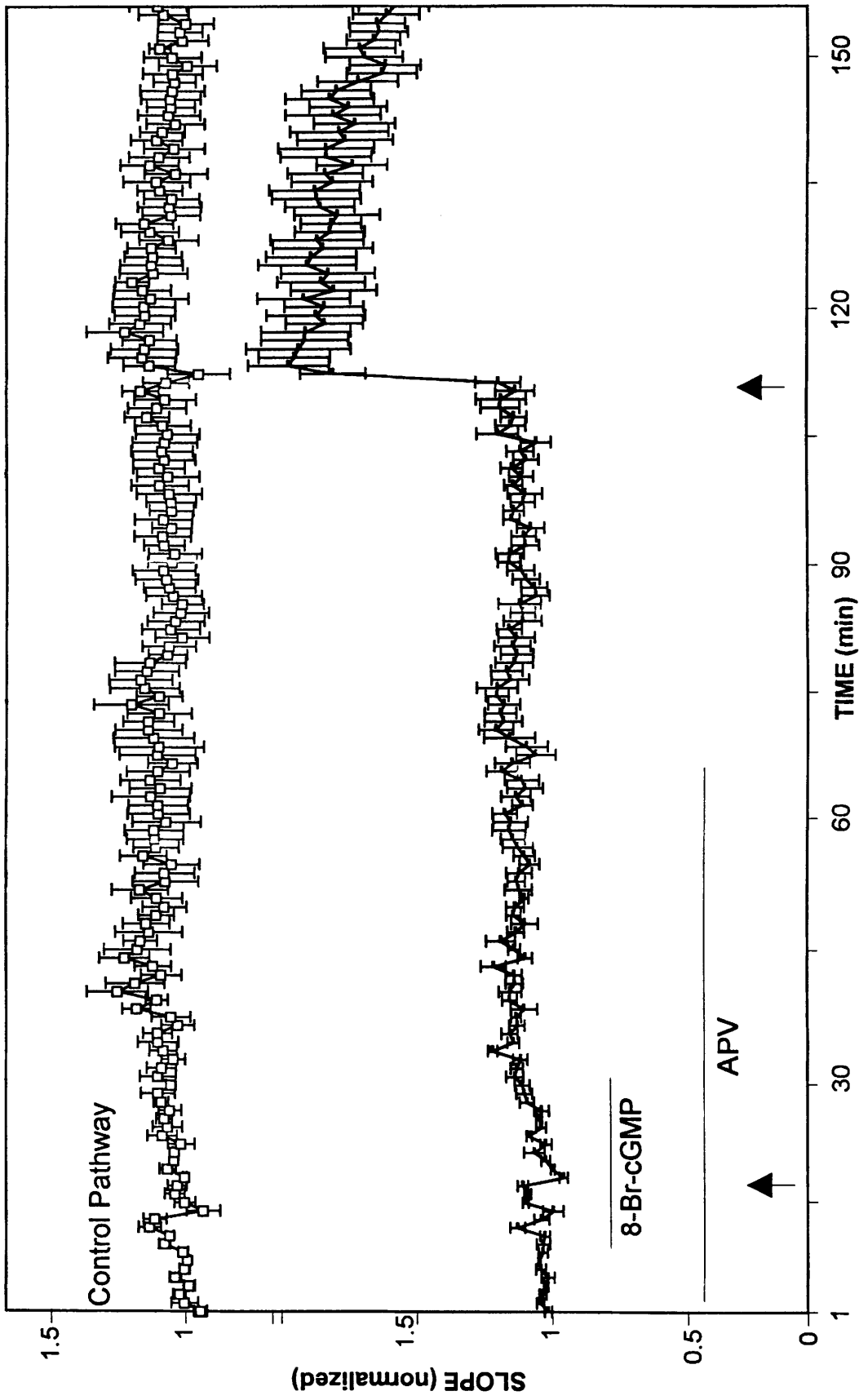
Figure 2: Results from the Boston group indicate that the block of LTP by APV is not overcome by 8-bromo-cGMP. Ensemble averages from five experiment monitoring synaptic strength versus time in experimental and control pathways. Slices were initially bathed in 50 μM D, L-APV. After a stable baseline was established, 100 μM 8-bromo-cGMP and DL_APV were washed out for 45 min. A100-Hz, 1-sec tetanus was administered to the control pathway (arrow), and robust LTP occurred.
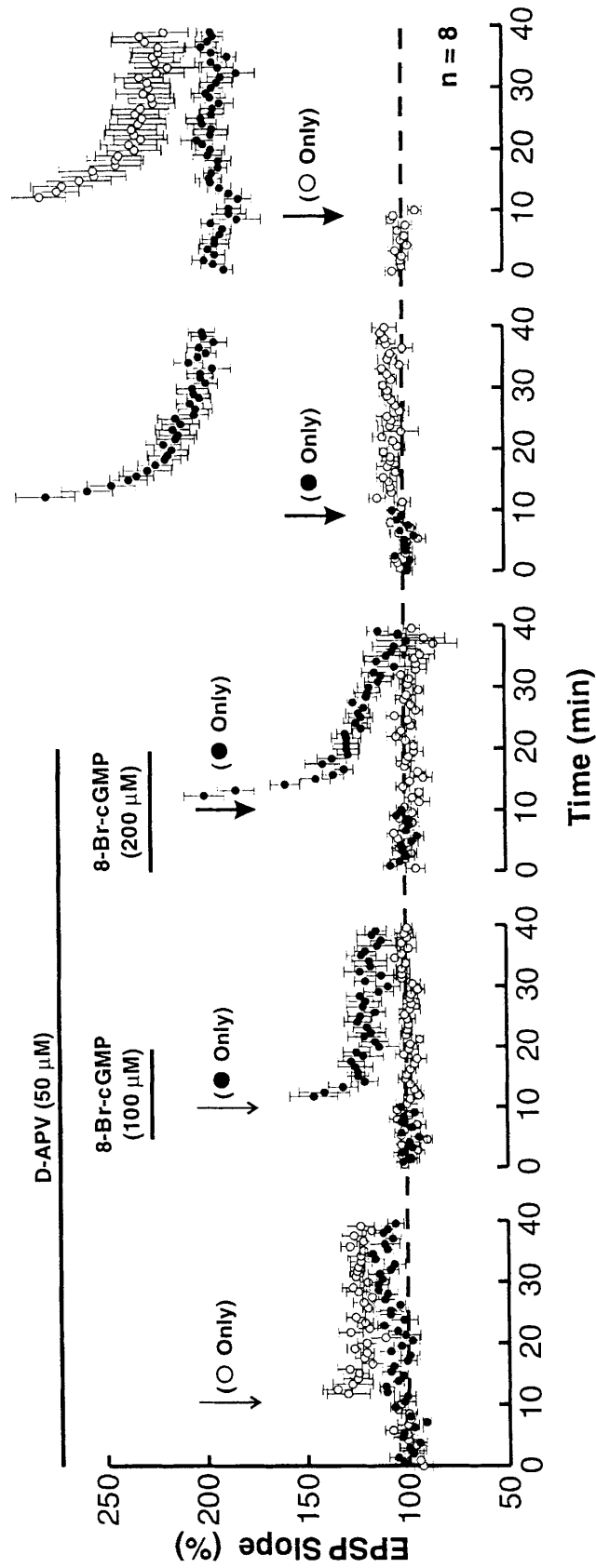
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Figure 3: Results from the San Francisco group indicate that block of LYTP by APV is not overcome by 8-bromo-cGMP. Ensemble averages from eight experiments monitoring synaptic strength in two independent inputs ($\bigcirc$ and $\bullet$). Slices were initially bathed in 50 μM D-APV. At the time indicated by the thin arrows, a 0.5-sec tetanus was given first to one input ($\bullet$). 8-Bromo-cGMP (100 μM) was them applied for a longer period (10 min), at which point a stronger tetanus (thick arrow, 100 Hz, 1 sec applied twice) still did not elicit LTP. Following washout of the D-APV and 8-bromo-cGMP, the same tetanus elicited a similar amount of LTP in both inputs. Each of the five different manipulations were performed in all eight slices. Prior to each manipulation, 10-min baselines were renormalized to 100%.

the tetanus was readministered and caused robust LTP ($64\pm2\%$ increase measured 30 min following the tetanus) (Fig. 2).

SAN FRANCISCO GROUP EXPERIMENTS

In this set of experiments, the experimental protocol was designed to mimic that of Zhuo et al. (1994) except that two independent inputs were used. These were alternately stimulated. Experiments were done on guinea pigs as in Zhuo et al. (1994). At the beginning of each experiment, D-APV (50 μM) was applied and a 10-min stable baseline was obtained. Because it has been reported that 50 μM D-APV may not completely prevent NMDA receptor activation during tetanic stimulation in guinea pig (Hanse and Gustafsson 1995), the tetanus (50 Hz, 0.5 sec) was first applied to one input in the absence of 8-bromo-cGMP (Fig. 3). This caused a modest but significant enhancement of synaptic strength when measured 20–30 min after the tetanus ($24\pm4\%$ increase, $n=8$). 8-Bromo-cGMP (100 M) was then applied for 5 min at which point the same tetanus was applied to the other input resulting in an increase in synaptic strength that was not significantly different from that observed in the absence of 8-bromo-cGMP ($17\pm6\%$, $P>0.3$, paired t-test; Fig. 3). To test whether the inability to generate LTP was attributable to inadequate levels of cGMP or insufficient activation of presynaptic terminals, a much stronger tetanus (100 Hz, 1 sec given twice separated by 20 sec) was subsequently given in the presence of a higher concentration of 8-bromo-cGMP (200 μM) applied 10 min before the tetanic stimulation. This pairing of strong tetanic stimulation with a high concentration of 8-bromo-cGMP still was insufficient to elicit LTP ($10\pm7\%$ increase). At the end of all of these experiments, the D-APV was washed out for 20–30 min and robust, stable LTP was generated (Fig. 3) by applying the strong tetanic stimulation first to the input that had just received this stimulation in the presence of D-APV ($100\pm7\%$ increase) and then to the other control input ($124\pm14\%$ increase).

Discussion

In this study three independent laboratories have examined the role of cGMP in generating LTP

by performing the critical experiment of pairing tetanic stimulation with bath application of 8-bromo-cGMP in the presence of APV. In contrast to previous reports (Zhuo et al. 1994; Arancio et al. 1995), this manipulation failed to generate LTP. These experiments were conducted in two different species (rat and guinea pig) using several different tetanic stimuli and the same or higher concentrations of 8-bromo-cGMP than had been used previously. The inability to generate LTP cannot be attributed to slice variability or poor health because all three groups found that the slices exhibited robust LTP following washout of the APV. The inability to generate LTP by pairing 8-bromo-cGMP with tetanic stimulation has also been reported by a fourth independent group (Schuman et al. 1994).

What could account for the differences between our findings and those of Zhuo et al. (1994)? Although we do not have a clear answer to this question, there are several possibilities. Subsequent to the publication of Zhuo et al. (1994), it was shown that tetanic stimulation in the presence of 50 μM APV can produce a small long-lasting synaptic enhancement (Hanse and Gustafsson 1995; Fig. 3) even without application of cGMP. This effect was not taken into consideration by Zhuo et al. (1994). A second possibility is that some potentiation may be explainable by the drift in baseline transmission evident in Figure 2d of Zhuo et al. (1994). A third possibility is that differences may stem from the fact that all of the present results were obtained using submerged slices, whereas Zhuo et al. (1994) used an interface recording chamber. A final possibility is that there are important differences in some unknown variable.

The experiments reported here do not provide any support for the proposal that an increase in presynaptic cGMP levels either alone or coupled with repetitive synaptic activation can produce potentiation or can overcome the block of LTP by APV. Thus, if retrograde messengers mediate the generation of LTP, some alternative intracellular cascade is presumably involved. Further work will be required to resolve this important issue.

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