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Long-term Increases in Excitability in the CA1 Region of Rat Hippocampus Induced by β-Adrenergic Stimulation: Possible Mediation by cAMP

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The cellular mechanisms underlying β-adrenergic potentiation in the CA1 region of the rat hippocampus were examined. A 10 min treatment with isoproterenol (ISO) induced a long-term depolarization of the pyramidal neurons that persisted for at least 30 min of washout; the ISO-induced decrease in the calcium-activated potassium conductance (afterhyperpolarization, or AHP) was similarly prolonged. The long-term excitability changes induced by ISO did not depend upon the calcium concentration of the medium and could be elicited in medium containing as little as 240 μM calcium. The persistent increase in population spike induced by ISO was mimicked by superfusion with several cAMP analogs and by forskolin (which directly activates adenylate cyclase), but not by the inactive dideoxyforskolin. Forskolin and cAMP analogs also induced decreases in AHPs that could be quite prolonged, but did not depolarize pyramidal neurons as consistently as did ISO. We hypothesize that activation of β-adrenergic receptors in the CA1 region of hippocampus may induce an alteration of the hippocampal "state" that can persist for as long as several hours, during which the induction of other forms of plasticity may be enhanced.

Although the role of norepinephrine (NE) as a putative transmitter in the CNS has been firmly established, its functional role as a neurotransmitter/neuromodulator remains somewhat poorly defined. At various times and in different brain regions, it has been described as an excitatory transmitter (Segal and Bloom, 1974a; Pang and Rose, 1987; Rose and Pang, 1989), as an inhibitory transmitter (Hoffer et al., 1971, 1973; Segal and Bloom, 1974a,b), as a modulator of GABAergic and glutamatergic transmission (Siggins et al., 1971; Freedman et al., 1977; Woodward et al., 1979), as an enabling factor in the development of synaptic plasticity (Pettigrew and Kasamatsu, 1978; Kasamatsu and Shirokawa, 1985), and as either a modulator or mediator of long-term potentiation (LTP) in the hippocampal formation (Hopkins and Johnston, 1984, 1988; Stanton and Sarvey, 1985a, 1987). In the CA1 region of the hippocampus, we have shown that NE will increase the amplitude of a synaptically evoked population spike via an interaction with a β-adrenergic receptor (Mueller et al., 1981). In previous studies it has been shown that the increases in population spike amplitude induced by β-adrenergic agonists can sometimes be very persistent (a phenomenon that we have termed β-adrenergic potentiation, or BAP), with a $t_{1/2}$ for decay back to baseline of approximately 2 hr (Kostopoulos et al., 1988; Heginbotham and Dunwiddie, 1991). Although we have ruled out trivial explanations for the relatively long duration of this response (e.g., persistence of agonist in the tissue), the mechanism underlying these long-term changes, and the locus at which these changes take place are unknown.

Previous studies have established that the short-term effects of NE that are mediated by β-adrenergic receptors occur as a result of increases in intracellular cAMP, which depolarizes pyramidal neurons and reduces the calcium-dependent potassium conductance (Madison and Nicoll, 1982, 1986a,b; Haas and Konnerth, 1983). However, long-term effects of β-adrenergic receptor activation such as we have described for population spike responses (Heginbotham and Dunwiddie, 1991) have not been previously described in intracellular recording studies. The persistent increases in the population spike amplitude that we observe could reflect changes in the resting membrane potential, changes in the threshold for activation of the sodium spike, or perhaps other changes such as the coupling between the dendritic site at which the EPSP is generated and the soma. In addition, the role played by cAMP in initiating the long-term response (BAP) is not known. Therefore, in the present studies we have investigated the cellular mechanism(s) underlying BAP and determined the site at which these changes occur. In particular, we have investigated the role played by intracellular second messengers such as calcium and cAMP in the initiation of this response, and characterized the intracellular changes that underlie the persistent increase in excitability.

Materials and Methods

Slices of rat hippocampus were prepared as described previously (Heginbotham and Dunwiddie, 1991), and intracellular and extracellular recordings were made from pyramidal cells in the CA1 region. Slices were superfused over both surfaces with oxygenated medium containing 124 mM NaCl, 25.7 mM $NaHCO_3$, 10 mM glucose, 3.3 mM KCl, 2.4 mM $MgSO_4$, 2.5 mM $CaCl_2$, and 1.2 mM KH_2PO_4, which was saturated with 95% O_2, 5% CO_2 and maintained at 33–34°C. In some experiments, the $CaCl_2$ concentration of the medium was changed to 1 or 5 mM with no change in the other ions. In other experiments, we characterized the effects of isoproterenol (ISO) on low-calcium-induced bursting; in these

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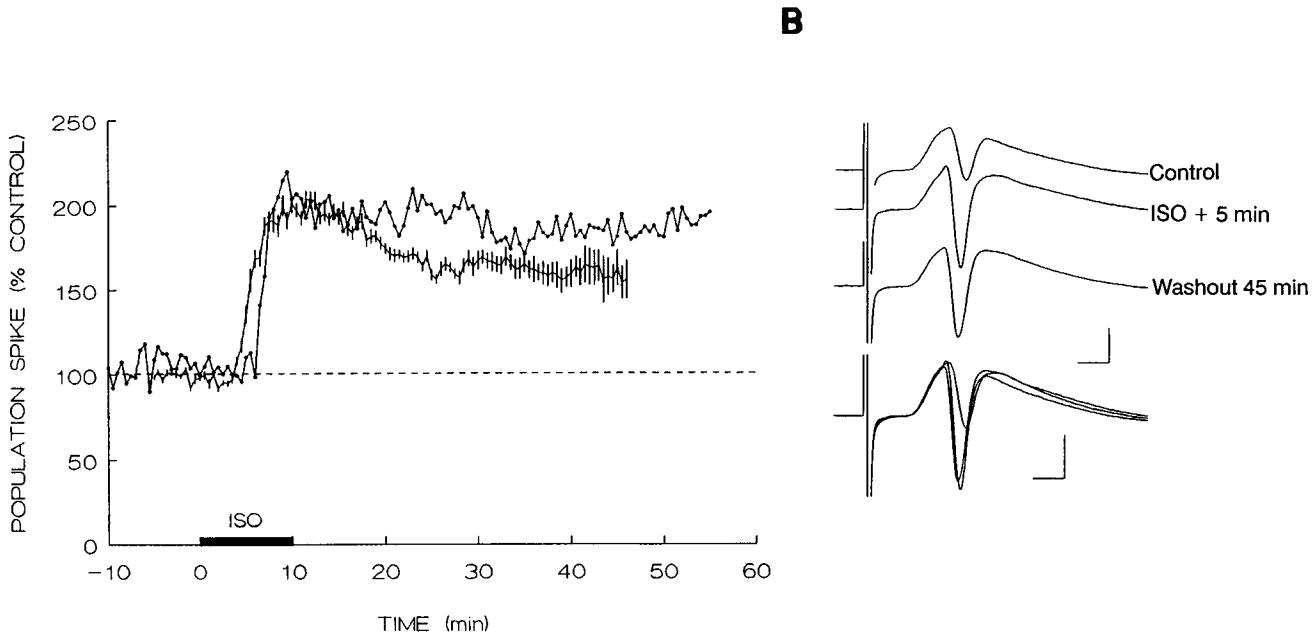

Figure 1. The effect of ISO on population spike amplitude. *A* illustrates the effect of superfusion with 500 nM ISO on the population spike response evoked from a single hippocampal brain slice (*line with circles*), and the mean ± SEM response from 13 slices treated with the same experimental protocol (*line with error bars*). As can be seen, ISO elicited an increase that far outlasted the 10 min period of superfusion with ISO, indicated by a *bar* along the x-axis. At 30 min of washout the increase was still 59 ± 8% above control ($p < 0.001$ for a paired t test vs. pre-ISO response). *B* illustrates signal averages of evoked responses obtained before, during, and after ISO superfusion, shown separately at the *top*, and superimposed at the *bottom*. The population spike response (negative-going potential) was markedly affected, but there was no change in the initial positive phase of the evoked response, which reflects primarily synaptic current. Calibration: 1 mV, 2 msec.

studies, the medium contained 0.24 mM $CaCl_2$ and 4 mM $MgSO_4$, with the other ions as in the control medium. Low-calcium bursting was evoked by stimulation of the fibers of the alveus, which elicits an initial antidromic population spike from the CA1 cell layer that is followed by repetitive decremental population spike responses (see, e.g., Figs. 2, 3). In the present studies, we measured the peak-to-peak amplitude of the first spike in the afterdischarge following the initial antidromic population spike response.

Intracellular recordings were made from slices maintained under conditions identical to the extracellular experiments. Putative pyramidal neurons were impaled with 50–80 MΩ glass microelectrodes filled with 2.5 M potassium acetate. If cells did not maintain a resting membrane potential of >55 mV, an input resistance of >25 MΩ, and at least 15 min of stable recording prior to ISO superfusion, they were not included in this experiment. Data from both the intracellular and the extracellular recordings were digitized and stored on a PC-based analysis system developed in this laboratory. Mean data are reported as the mean ± the standard error, and statistical significance was determined with a two-tailed Student's t test, a paired t test, or a χ^2 test as appropriate. Differences were considered statistically significant if $p < 0.05$.

Drugs. L-Isoproterenol (ISO), 8-bromo-cyclic AMP (br-cAMP), 8-*p*-chlorophenylthio-cyclic AMP (pcpt-cAMP), dibutyryl-cyclic AMP, and forskolin were obtained from Sigma Chemicals, dideoxyforskolin from Calbiochem, cyclopentyltheophylline (CPT) from Research Biochemicals Inc., and 8-methylthio-cyclic AMP (mt-cAMP) from ICN Pharmaceuticals. Drugs were made up as stock solutions at ≥100× the final concentration desired and added to a constant flow of superfusion fluid with a Sage syringe pump. A previous study has shown that the <1% dilution of the medium has no effect on responses evoked in the slices (Mueller et al., 1981). Because of the limited solubility of forskolin, it was made up as a 50 mM stock solution in 100% dimethyl sulfoxide, diluted 1:5000 or 1:50,000 in oxygenated artificial cerebrospinal fluid (ACSF), and injected directly into the slice chamber rather than being added to the flow of control medium. In all experiments, each drug application was preceded by a control period of at least 10 min. ISO superfusion typically lasted 10 min and was followed by a ≥30 min wash period, whereas forskolin and cyclic AMP analogs were superfused for at least 15 min.

Results

Effects of ISO on population spikes

When hippocampal slices were superfused with 500 nM ISO, a consistent increase in the amplitude of the evoked population spike response was observed, as has been reported previously (Mueller et al., 1981). However, the increases in many cases markedly outlasted the duration of the superfusion with ISO, as is illustrated in Figure 1. The mean population spike amplitude was still significantly potentiated following 30 min of washout, and the magnitude of the increase was quite comparable to what we have reported previously (Heginbotham and Dunwiddie, 1991). We have termed the long-term component of the change in the evoked population spike response BAP (β-adrenergic potentiation). No changes were seen in the slope of the rising phase of the field potential recorded from the cell body layer (Fig. 1*B*), suggesting that these responses primarily reflected changes in cellular firing that were not accompanied by increases in synaptic transmission (see also Haas, 1986; Heginbotham and Dunwiddie, 1991).

Involvement of calcium in BAP

Because other forms of hippocampal plasticity such as LTP are dependent upon extracellular calcium, we determined whether BAP could be influenced by varying the calcium concentration of the medium. Doubling the calcium concentration to 5.0 mM did not significantly change either the short-term effects of ISO or the persistent effects recorded after 30 min of washout (Table 1, Fig. 2). Decreasing the calcium concentration to 1.0 mM significantly reduced the fraction of slices that showed BAP (2 of 12 vs. 9 of 15 in control medium; $\chi^2 = 9.39$; df = 11; $p <$

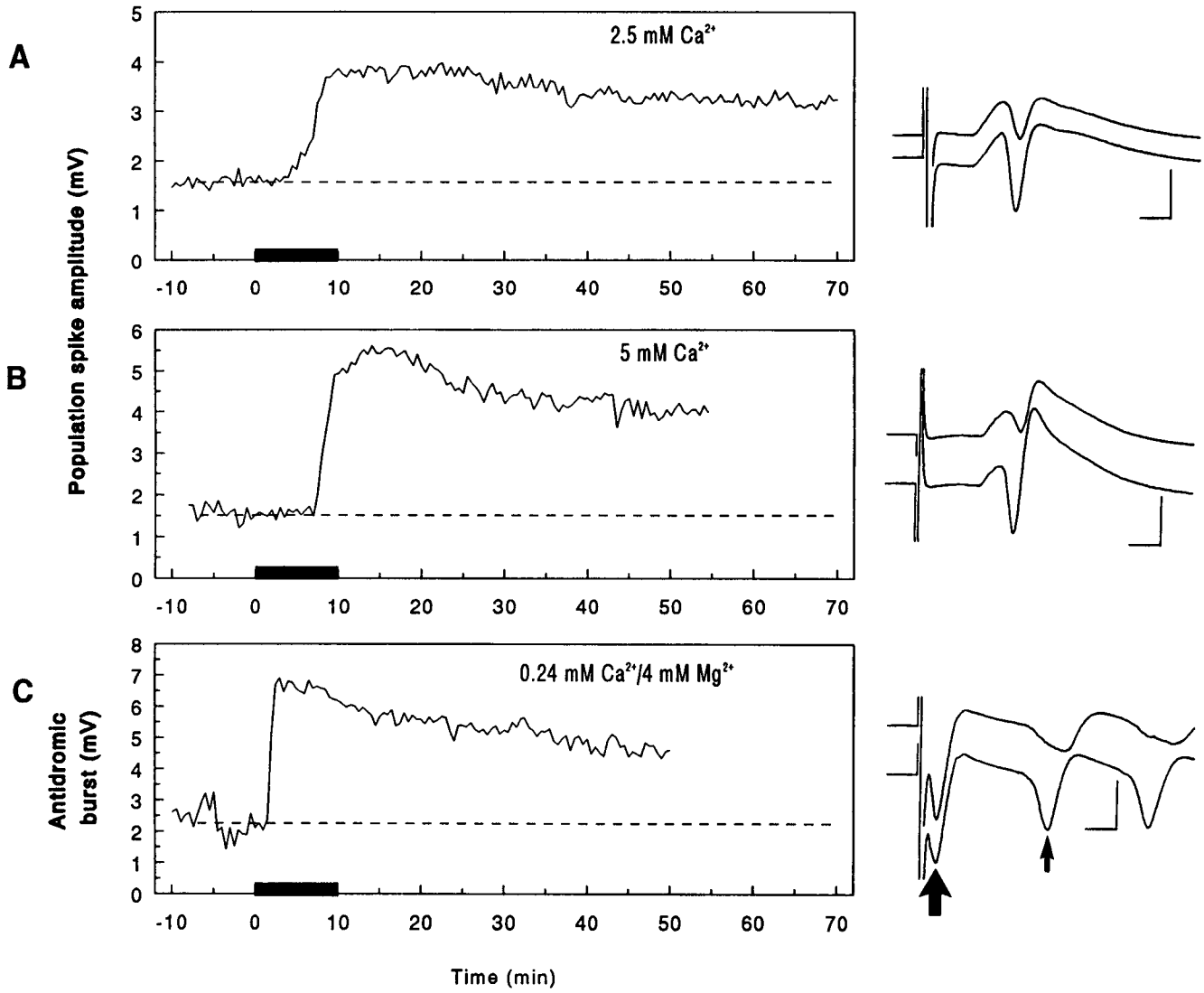

Figure 2. The effect of calcium on BAP. Each panel shows the effect of superfusion with 500 nM ISO (*bars* along x-axis) in medium containing the indicated concentrations of Ca^{2+}. *A* is an example of the response in normal control medium containing 2.5 mM Ca^{2+} and 2.4 mM Mg^{2+}. *B* illustrates a similar response to ISO in a slice maintained in 5 mM Ca^{2+} and 2.4 mM Mg^{2+}. Both the acute and long-term response to ISO are apparent in this slice. In *C*, a similar persistent response to ISO is observed in medium containing 0.24 mM Ca^{2+} and 4 mM Mg^{2+}. The response measure was the amplitude of the first afterdischarge evoked by antidromic stimulation of the pyramidal neurons (the initial antidromic population spike is denoted by the *large arrow* in the records to the *right*, and the first afterdischarge, by the *small arrow*). The averaged records show evoked responses before and 30 min after superfusion with 500 nM ISO. Calibration: *A* and *B*, 2 mV, 2 msec; *C*, 4 mV, 2 msec.

0.005), but under these conditions we found it to be extremely difficult to maintain stable baseline responses, and it seemed possible that the frequent failure of BAP under these conditions was related to a long-term decline in the synaptic response. For these reasons, we determined whether BAP could be observed when testing a completely different kind of response, namely, low-calcium-induced bursting. When slices are maintained in medium containing 0.24 mM calcium and 4 mM magnesium, antidromic stimulation of the axons of CA1 pyramidal neurons evokes an initial antidromic population spike (which is unaffected by drug treatment), which is followed by decremental repetitive afterdischarges (Fig. 2*C*; see also Schubert and Lee, 1986; Dunwiddie and Fredholm, 1989). The amplitude of these afterdischarges is sensitive to agents that alter the excitability of the pyramidal neurons. When low-calcium-induced bursting was tested in slices superfused with 500 nM ISO, we observed that the amplitude of the afterdischarge was significantly increased and that this increase was maintained following washout of ISO with a time course that was virtually identical to the changes in the synaptic responses following ISO (compare Figs. 1, 3; see also Table 1).

Site of ISO action

The increase in the population spike response with no change in the field EPSP (fEPSP), and the observation that long-term increases could be observed under conditions where calcium-dependent transmitter release was completely blocked, suggested that the mechanism underlying this response might be a postsynaptic change in the excitability of the pyramidal neurons. To determine whether this was the case, we characterized the long-term effects of ISO using intracellular recording from CA1 pyramidal neurons. Activation of β-adrenergic receptors has been reported to depolarize the resting membrane potential and to reduce the afterhyperpolarization (AHP) that is activated by

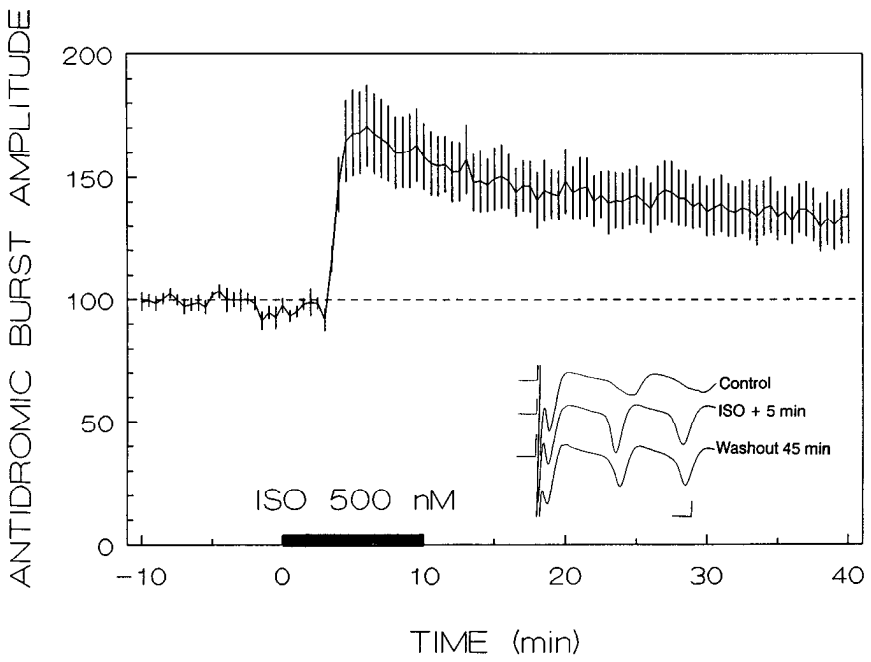

Figure 3. The effect of ISO on low-calcium bursting. The mean ± SEM increase in the initial afterdischarge is shown in response to superfusion with 500 nM ISO is shown as a percent of control. These results are for a group of 12 slices treated with an identical protocol. As can be seen from the averaged responses (*inset*), ISO had no effect upon the antidromic population spike (initial negativity), but induced a marked increase in the amplitude of the primary afterdischarge. Each response is the average of five evoked responses. Calibration: 2 mV, 2 msec.

depolarizing current injection (Madison and Nicoll, 1982, 1986a; Haas and Konnerth, 1983). In the present experiments, we examined the effect of ISO on both of these parameters. ISO induced a significant depolarization of the resting membrane potential that reached a peak of approximately 5–6 mV (Fig. 4; see also Table 2) but was not accompanied by a significant change in the input resistance. Although the membrane repolarized to some extent following ISO washout, it remained significantly depolarized for at least 30 min following washout of ISO ($p < 0.05$, paired t test of pre-ISO vs. 30 min responses). As described in previous studies, we found that the AHP was markedly attenuated by ISO (Fig. 4, Table 2). Because ISO-induced depolarization would increase the amplitude of the AHP, in some cells the change in the resting membrane potential was offset by injection of constant current through the recording electrode. Significant acute changes in the AHP were observed regardless of whether or not the cells were "clamped" in this manner. The decrease in the AHP also was maintained in the "unclamped" cells for at least 30 min of washout (Table 2). The change in the AHP at 30 min was not statistically significant in the group of three cells that were manually "clamped," but was significant in the group of five cells that were not clamped, and when the two groups were pooled (Table 2; $p < 0.01$, two-tailed paired Student's t test). The input resistance (determined by the

Table 1. Effects of ISO, cAMP analogs, and forskolin on population spike amplitude

Treatment	Pretreatment	Acute effect (percentage increase above control)	30 min effect (percentage increase above control)
Isoproterenol, 500 nM	None	134 ± 13 (24)***	84 ± 12 (24)***
	5.0 mM calcium medium	160 ± 11 (12)***	81 ± 17 (12)***
	0.24 mM calcium medium[a]	71 ± 16 (12)**	34 ± 11 (12)*
	pcpt-cAMP, 100 μM	4.0 ± 4.8 (12)	
cAMP analogs			
8-methylthio-cAMP, 250 μM	None	79 ± 14 (8)***	55 ± 12 (8)**
dibutyryl-cAMP, 250 μM	None	97 ± 17 (8)***	64 ± 14 (8)**
8-bromo-cAMP, 500 μM	None	−45 ± 14 (6)*	58 ± 8 (6)**
	CPT, 100 nM	208 ± 50 (7)**	91 ± 24 (7)**
pcpt-cAMP, 100 μM	None	133 ± 24 (9)***	−16 ± 9 (9)
	Theophylline, 250 μM	216 ± 13 (7)***	16 ± 5.5 (7)
Forskolin, 10 μM	None	94 ± 22 (7)*[b]	86 ± 12 (15)***
Dideoxy-forskolin, 10 μM	None	24 ± 8.5 (6)*	20 ± 10 (6)

Numbers in parentheses indicate the number of slices tested. All comparisons made using a paired Student's t test.

* $p < 0.05$.

** $p < 0.01$.

*** $p < 0.001$.

[a] Low-calcium burst response.

[b] Average is only for those slices which did not show acute decreases.

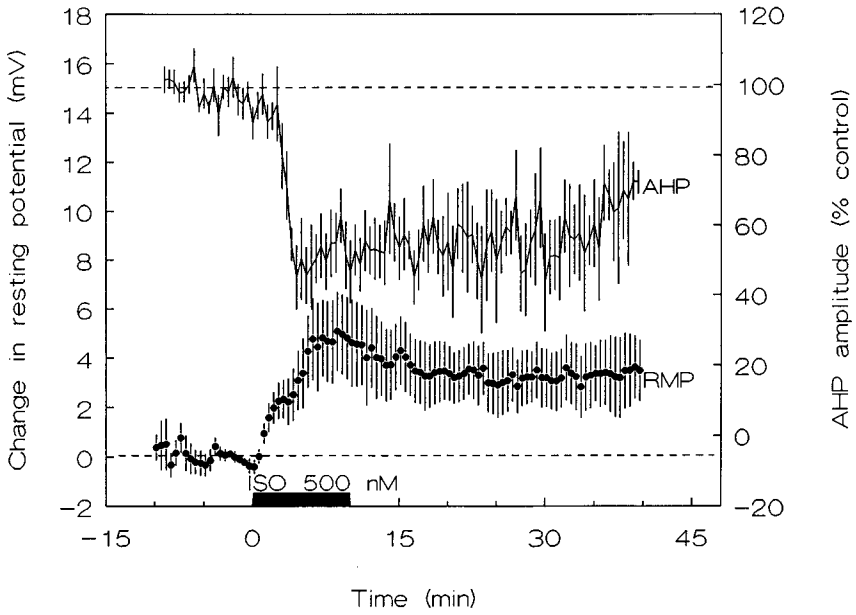

Figure 4. Effect of ISO on resting membrane potential (RMP; *line with circles*) and AHP (*line with no symbols*). The effect of superfusion with 500 nM ISO is shown for six cells that had stable pre-ISO responses and that were followed for at least 30 min of washout following ISO superfusion. The mean RMP was 63 ± 1.7 mV for these cells prior to ISO, and the change in the RMP (*left scale*) is shown normalized to each cell's pre-ISO baseline. The AHP responses (*right scale*) were evoked by depolarizing current injection, were measured at the peak of the AHP, and were normalized to the pre-ISO baseline. Because the number of spikes evoked by the depolarizing current injection usually increased following ISO, the current was reduced to equate the number of spikes evoked in the two conditions.

injection of hyperpolarizing current) was not significantly affected by ISO. As was suggested by the extracellular recording studies, EPSPs were not significantly increased by ISO, and the overall decreases that were observed clearly could not support *increases* in the population spike response (Table 2). The amplitudes of synaptically evoked IPSPs were increased by 12% acutely, which again could not explain increases in evoked responses, but there was a 27% reduction following 30 min of drug washout (Table 2). A decrease in IPSPs could provide a possible explanation for increases in population spikes at this later time point.

Role of cAMP in responses to ISO

Previous studies have suggested that the acute effects of ISO are mediated via increases in cAMP, because the effects of ISO can be mimicked by cAMP analogs and by forskolin (Mueller et al.,

Table 2. Effects of ISO, forskolin, and cAMP analogs on intracellularly recorded parameters

Response measure/ treatment	Acute effect	30 min effect
Depolarization of resting membrane potential (mV)		
ISO, 500 nM	6.4 ± 1.0 mV (10)***	3.5 ± 1.2 mV (6)*
Forskolin, 10 μM	2.5 ± 1.4 mV (8)	
pcpt-cAMP/br-cAMP	−0.5 ± 0.2 mV (5)	
AHP (percent control)		
ISO, 500 nM[a]	32 ± 9% (8)***	52 ± 14% (5)*
ISO, 500 nM	39 ± 7% (7)***	76 ± 27% (3)
Forskolin, 10 μM	46 ± 19% (8)*	
pcpt-cAMP/br-cAMP	33 ± 10% (6)**	
EPSP amplitude (percent control)		
ISO, 500 nM[a]	77 ± 14% (6)	67 ± 17% (3)
Forskolin, 10 μM[a]	107 ± 10% (6)	
pcpt-cAMP/br-cAMP[a]	124 ± 21% (5)	
Change in input resistance (MΩ)		
ISO, 500 nM	0.33 ± 0.62 MΩ (14)	0.52 ± 1.25 MΩ (9)
Forskolin, 10 μM	−1.77 ± 0.9 MΩ (9)	
pcpt-cAMP/br-cAMP	−0.77 ± 0.68 MΩ (6)	
$GABA_A$ IPSP (percent control)		
ISO, 500 nM[a]	112 ± 4.4% (8)*	73 ± 21 (6)
Forskolin, 10 μM[a]	69 ± 19% (4)	
pcpt-cAMP/br-cAMP[a]	108.8 ± 15% (6)	

* $p < 0.05$, paired Student's t test.

** $p < 0.01$, paired Student's t test.

*** $p < 0.001$, paired Student's t test.

[a] Average is only for those neurons where membrane potential was manually "clamped" and depolarizing current pulse was unchanged. Data for cAMP analogs did not show differences and were pooled; pcpt-cAMP was 100 μM; br-cAMP, 500 μM in the presence of 100 nM CPT; $n = 3$ for each.

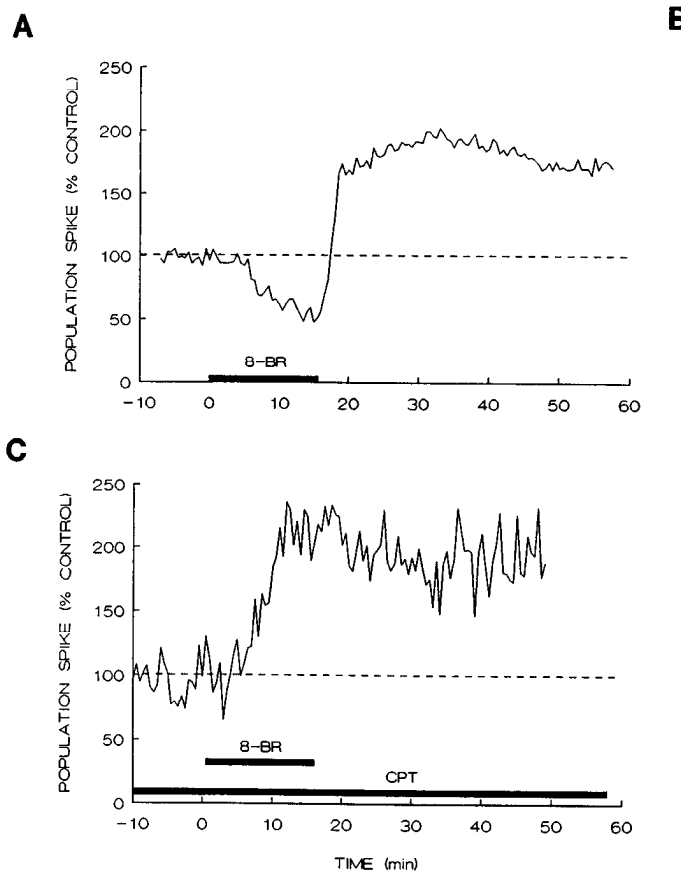

Figure 5. Effect of br-cAMP on population spike responses. Responses to br-cAMP (*8-Br*) in two different slices are shown in *A* and *C*. In both cases, the slice was superfused with 500 μM br-cAMP for 15 min, but in *C* the slice was pretreated with 100 nM CPT, a selective adenosine A1 receptor antagonist, for 40 min prior to as well as during and after br-cAMP superfusion. Drug superfusion is indicated by the *bars* along the x-axis. Signal-averaged evoked responses from the slices shown in *A* and *C* are shown in *B* and *D*, respectively. The *top trace* of each group is from the pre–br-cAMP period; the *middle trace,* at the end of the br-cAMP superfusion; and the *last record,* after 30 min of washout. All three traces are superimposed at the *bottom* at higher gain. Note that in *B* there is a small decrease in the rising phase of the population response in the presence of br-cAMP (*middle record*), indicative of a decrease in the fEPSP response, which is consistent with an activation of adenosine receptors by br-cAMP (Dunwiddie and Hoffer, 1980). Calibration: 1 mV, 2 msec.

1981; Madison and Nicoll, 1986a,b). However, the role of cAMP in BAP has not been determined. In the first set of experiments, we investigated the effects of superfusion with cAMP analogs on evoked field responses; four different analogs were tested to try to identify common actions [presumably mediated via protein kinase A (PKA) stimulation] and to identify other nonspecific effects that are not mediated via this mechanism. With each analog, we initially tested a range of concentrations to obtain a rough estimate of potency and then tested a number of slices at a single concentration that reliably elicited increases in the population spike response comparable to those observed with ISO (see Table 1). The effects of br-cAMP on the evoked population spike response are illustrated in Figure 5. Although superfusion with 500 μM br-cAMP did cause a long-term increase in the population spike response, the acute effect was a decrease in this potential (Fig. 5*A*). Because we have previously observed that cAMP analogs as well as cAMP itself can depress synaptic transmission by activating adenosine receptors (Mueller et al., 1981), we repeated these experiments in the presence of 100 nM CPT, a selective adenosine A1 receptor antagonist (Dunwiddie and Fredholm, 1989). Under these conditions, br-cAMP elicited both acute and long-term increases in the population spike response that were qualitatively similar to the response to ISO (Fig. 5*C*). Two other cAMP analogs (mt-cAMP and dibutyryl-cAMP) both induced acute increases in the population spike response, even without an adenosine receptor antagonist, and these effects persisted for at least 30 min following washout. The results of experiments with these analogs are summarized in Table 1. As with ISO, the population spike response was increased with each of these analogs without a corresponding increase in the amplitude of the fEPSP response.

The only cAMP analog tested that produced a qualitatively different result was pcpt-cAMP. While this analog mimicked the acute increase in the population spike response observed with ISO, it did not produce a significant long-term increase in the population spike response (Fig. 6). Although this analog did not have a direct depressant effect by itself as did br-cAMP, the acute increase in the population spike response was significantly potentiated by pretreatment with the adenosine receptor antagonist theophylline (250 μM), suggesting that there may have been an underlying depressant component of the response as well. Nevertheless, even under these conditions the population spike response returned to baseline following 20–30 min of washout.

A possible explanation for these results would be that the increases in the population spike response induced by pcpt-cAMP were not mediated via effects on PKA, but rather through some other "nonspecific" action of this analog. We have shown, for example, that this analog appears to have direct antagonistic effects upon GABA receptors, albeit in somewhat higher concentrations (Leidenheimer et al., 1990). Therefore, we determined whether increases in the population spike induced by pcpt-cAMP or br-cAMP would occlude subsequent responses to ISO, as would be expected if they shared a common mechanism of action. If these analogs did not activate PKA in the concentrations used in these experiments but increased the population spikes via different mechanisms of action, then we would predict that their effects should be additive with those of ISO. In these experiments, we pretreated slices either with 3 mM br-cAMP or with 100 μM pcpt-cAMP (which increased the population spike amplitude), then reduced the stimulation intensity so that the population spike amplitude was returned to the original baseline (see Fig. 7*C*), and then tested for ISO sensitiv-

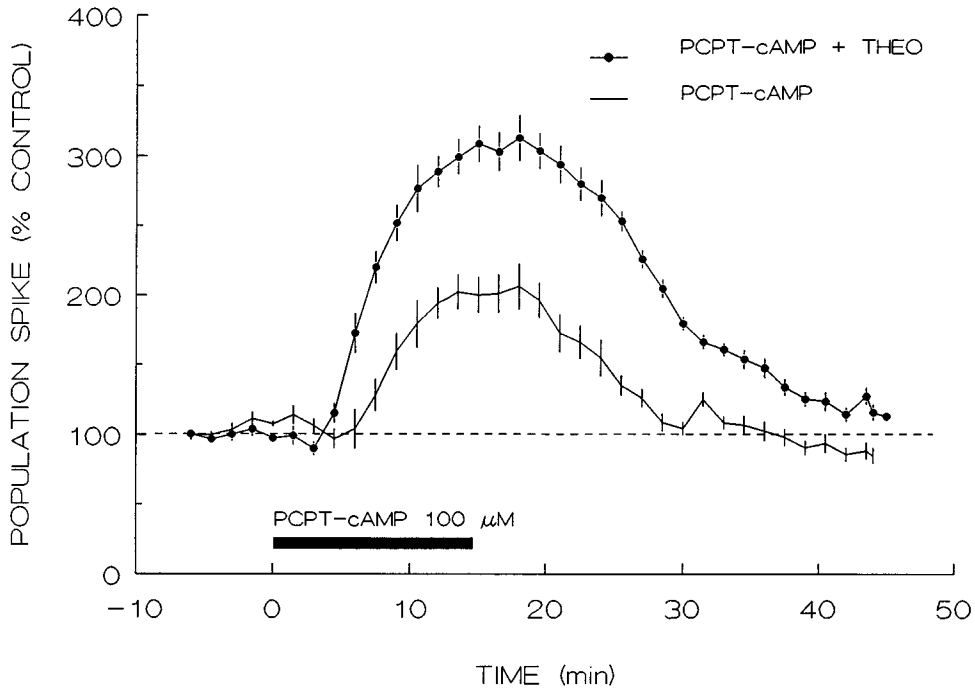

Figure 6. Effect of pcpt-cAMP on the population spike response. Slices were superfused with either 100 μM pcpt-cAMP alone (*PCPT-cAMP*; $N = 9$), indicated by the *horizontal bar*, or pretreated with 250 μM theophylline and then tested with this cAMP analog (*+THEO*; $N = 7$). Each *point* is shown as the mean ± SEM for all the slices tested.

ity. As shown in Figure 7, *A* and *C*, pretreatment with either cAMP analog completely inhibited the normal response to ISO. In a group of 12 slices pretreated with 100 μM pcpt-cAMP, the acute response to ISO was completely blocked (Table 1; $p < 0.001$ vs. control). As a second means of determining whether the effects of pcpt-cAMP were mediated via PKA, we attempted to block its actions with protein kinase inhibitors. However, at a concentration of 100 μM, neither 1-(5-isoquinolinesulfonyl)-2-methylpiperazine (H-7) nor *N*-[2-(methylamino)-ethyl]-5-isoquinoline-sulfonamide (H-8) blocked responses to either ISO or pcpt-cAMP (0 of 12 slices for ISO, 1 of 14 slices for pcpt-cAMP). With higher concentrations of these inhibitors, it was not possible to maintain synaptic responses. Our laboratory, as well as others (Muller et al., 1988; Corradetti et al., 1989; Leidenheimer et al., 1990), has shown that these agents have profound direct excitatory effects upon pyramidal neurons that do not seem to be mediated via PKA interactions, but rather by a direct antagonism of $GABA_A$ receptors. For this reason, we could not use this approach to determine the role of PKA in responses to pcpt-cAMP.

As a second means of testing for the involvement of cAMP in BAP, we superfused slices with 1–10 μM forskolin, which directly activates adenylate cyclase and increases cAMP levels (Seamon et al., 1981). Forskolin also reproduced the long-term effects of ISO, both qualitatively and quantitatively, by causing persistent increases in the population spike response without affecting the fEPSP response (Fig. 8). However, the short-term effects of forskolin were much more inconsistent. In 8 of 15 slices treated with 10 μM forskolin, there was an initial increase in the population spike response followed by a complete loss of this response, during which time the fEPSP response was markedly reduced. Upon washout, the population spike response returned and stabilized at a persistently elevated level. The other 7 slices responded only with increases in the population spike response (Fig. 8), as was seen with ISO. The occurrence of a transient period of depression in some of the slices did not affect the magnitude of the long-term increase in the population spike measured following 30 min of washout [85 ± 17% increase in the transient depression group ($n = 8$) vs. 86 ± 17% increase in the group that was not depressed ($n = 7$)]. The inactive analog of forskolin, dideoxyforskolin, produced only a modest increase in the evoked population spike response that was statistically significant at 0 min washout but not at 30 min (Table 1).

As a final means of determining the role of cAMP in BAP, we compared the effects of cAMP analogs and forskolin to the effect of ISO on intracellularly recorded responses. In terms of acute effects, ISO, forskolin, pcpt-cAMP, and br-cAMP all inhibited AHP responses induced by depolarizing current injection by over 50% (Table 2; the responses to pcpt-cAMP and br-cAMP did not differ, so they were combined in the data in Table 2). In cells from which stable long-term recordings could be made, we often saw a marked persistence of the effects of both forskolin and the cAMP analogs; several such responses are illustrated in Figure 9. However, unlike the situation with ISO, there are no "antagonists" for these effects of cAMP analogs and forskolin; for reasons discussed above, we could not use kinase inhibitors for this purpose. In light of this, it is difficult to determine whether the persistence of the effects of cAMP analogs and forskolin reflects a long-term change that is triggered by brief (10 min) exposure to these agents, or whether the prolonged response simply reflects a slow washout of these drugs from the tissue. Unlike ISO, which produced a significant and persistent depolarization of pyramidal neurons, neither forskolin nor the cAMP analogs had consistent effects on resting membrane potentials (Table 2). Other cellular parameters such as input resistance and the amplitudes of EPSPs and IPSPs were also not affected by forskolin or cAMP analogs.

Discussion

The present experiments demonstrate that in the CA1 region of the rat hippocampus, ISO can act upon β-adrenergic receptors to induce a long-term increase in the evoked population spike

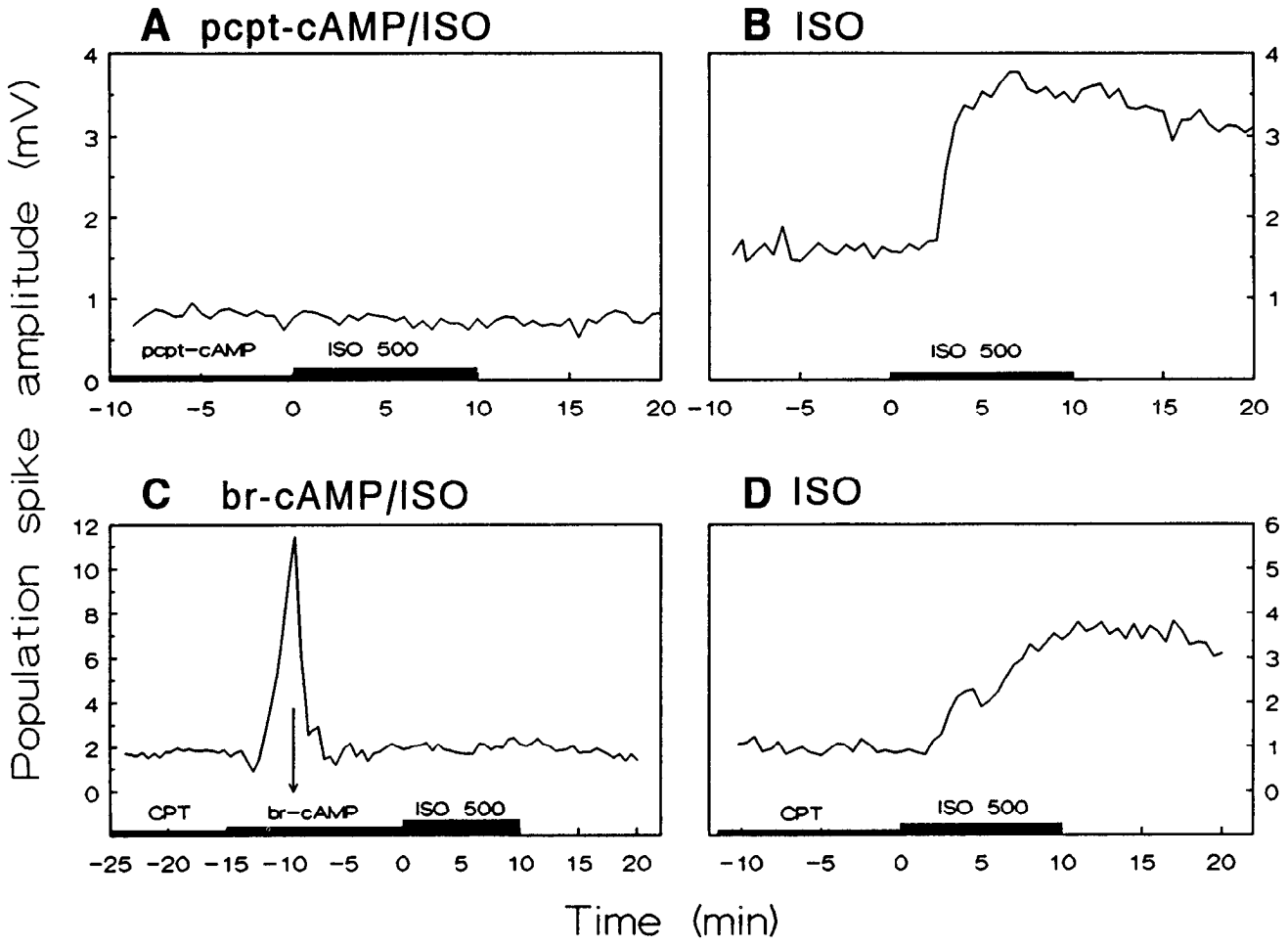

Figure 7. Effect of pretreatment with cAMP analogs on ISO responses. Slices either received no pretreatment (*B* and *D*) or were pretreated with 100 μM pcpt-cAMP (*A*) or 3 mM br-cAMP (*C*), prior to testing with 500 nM ISO. In *A*, the pcpt-cAMP pretreatment began before the beginning of the data shown. *B* illustrates a normal response to ISO obtained from another slice prepared from the same hippocampus. In *C*, the br-cAMP superfusion was begun at −14 min and produced an approximate fivefold increase in the population spike response; the stimulation voltage was then reduced (*arrow*) to return the baseline response to its original value. This slice was also superfused with 100 nM CPT from 40 min prior to the ISO superfusion to block the effects of br-cAMP mediated via adenosine receptors (see Fig. 5). *D* illustrates a paired ISO control response from an untreated slice obtained from the same animal.

response. Several experimental findings suggest that, as with the acute effects of β-adrenergic receptor activation, increases in intracellular cAMP mediate this response. The fact that forskolin, several cAMP analogs, and histamine (Kostopoulos et al., 1988) all elicit a long-term increase in the population spike response suggests that it is the increase in cAMP (and presumably PKA activation) rather than activation of β-adrenergic receptors per se, that results in the long-term increase. While the present studies clearly implicate cAMP as a second messenger in this response, they do not provide strong support for an involvement of calcium channels or calcium influx in the induction of BAP. Although we observed a reduction in BAP with medium containing 1 mM calcium (as opposed to 2.5 and 5.0 mM calcium), the studies involving low-calcium bursting, which were carried out in medium containing 0.24 mM calcium, demonstrated that persistent increases in afterdischarge amplitude could be induced in the virtual absence of extracellular calcium. Unless there are two separate processes, a calcium-dependent one that mediates BAP and a second calcium-independent process that underlies long-term increases in antidromic bursting, the simplest conclusion is that the persistent changes that we describe are independent of extracellular calcium.

The intracellular recording studies demonstrate that the depolarization of the pyramidal neurons induced by ISO is persistent, and this depolarization would provide a mechanistic basis for the increases in the population spike responses observed in the extracellular recording experiments. Comparable changes in the membrane potential have been described by other groups, although no mention is made of whether these effects were persistent, and in some cases they were clearly reversed upon washing. However, Stanton et al. (1989) reported depolarizations in the dentate gyrus following superfusion with NE that persisted for an average of 93 min following washout. These results suggest that persistent depolarization may occur in several types of hippocampal neurons following β-adrenergic receptor activation. Further studies will be required to determine the extent to which these processes share common mechanisms.

Unlike ISO, neither forskolin nor the cAMP analogs produced statistically significant depolarizing responses in our experiments, although some previous studies have found such effects

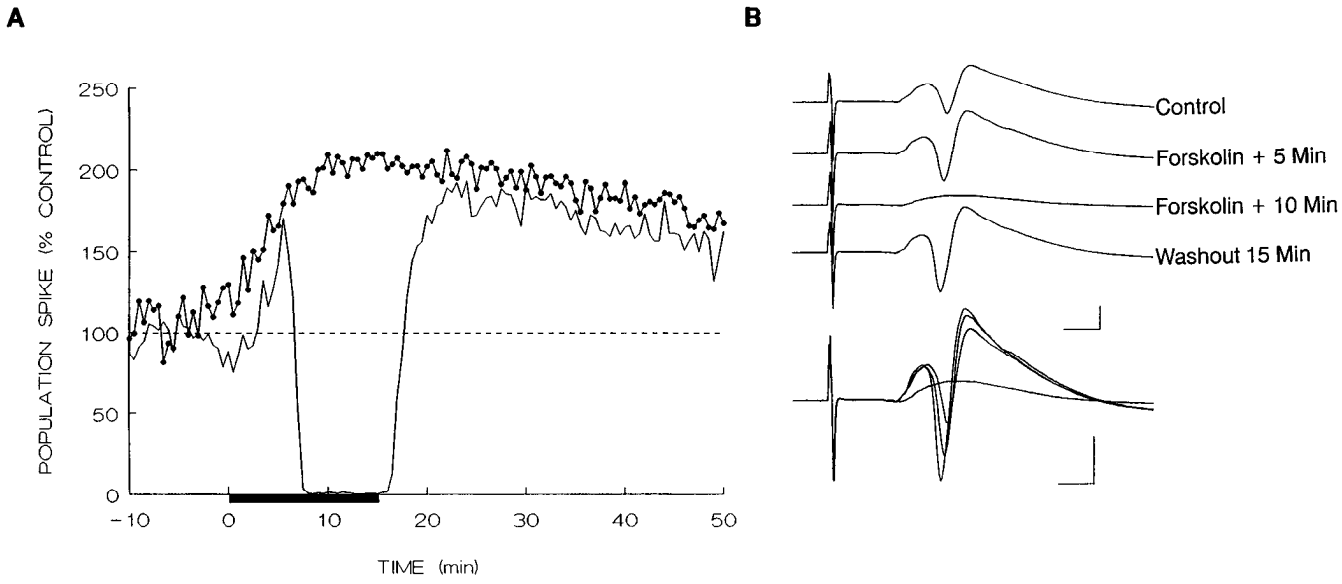

Figure 8. Effect of forskolin on the population spike response. *A* shows the effect of superfusion with 10 μM forskolin (*horizontal bar*) on two individual slices; each *line* corresponds to a different slice. The slice illustrated by the *line with symbols* showed no depressant effects of forskolin, whereas the *line without symbols* shows the transient depression observed in 8 of 15 slices. Averaged responses from another such slice showing the transient depression are shown in *B*; as can be seen, the increase in the population spike response was not accompanied by a corresponding change in the rising phase of the field response recorded in the cell body layer, suggesting that there was no effect upon the fEPSP. However, the decrease in the population spike was accompanied by a marked decrease in the slope of the rising phase of the field response, indicating that synaptic transmission was directly affected by forskolin. Calibration: 1.5 mV, 2 msec.

(Madison and Nicoll, 1986b). Some individual slices in our experiments showed either depolarizations in the membrane potential or increases in hyperpolarizing current required to clamp the membrane potential that were time-locked to the onset of drug superfusión, but overall there was no significant change in this parameter. The reasons for this are not entirely clear but may relate to the condition of the cell that is being recorded from, and possibly its basal levels of calcium (see below).

One intracellular response that has been consistently observed in all studies including our own is a decrease in the AHP that follows depolarizing current injection. This effect is observed with ISO, with cAMP analogs, and with forskolin, and under the conditions used in this study, these effects were quite prolonged. Although we could not rule out the possibility that the persistent effects of cAMP analogs and forskolin reflected slow washout, these findings are at least consistent with the hypothesis that activation of PKA by cAMP or cAMP analogs results in prolonged changes in the AHP. It is possible that this response underlies the changes in population spike responses as well; if a calcium-dependent potassium conductance like the one underlying the AHP is active under resting conditions (i.e., it shows some low level of tonic activity with normal intracellular calcium concentrations), then inhibiting this conductance would result in a depolarization as well as some increase in input resistance. If the reduction in this conductance is the primary effect of activation of PKA, this might also provide an explanation for why we did not always observe membrane depolarizations (only 6 of 14 cells with cAMP analogs and forskolin) and increases in input resistance (5 of 14), whereas all showed reduced AHPs (14 of 14). If the depolarization depends upon blocking a tonic calcium-activated potassium conductance, then it would only be observed in cells where this conductance makes a substantial contribution to the resting membrane potential, whereas the AHP would be affected in every cell because it is the primary target of PKA activation.

Although the depolarization of the pyramidal neurons and/or the decrease in AHPs provides a mechanism for BAP at the cellular level, the reason for its persistence is unclear. Previous studies have demonstrated that this phenomenon cannot be explained simply on the basis of prolonged occupation of β-adrenergic receptors by ISO, because the effect cannot be reversed by β-adrenergic antagonists (Kostopoulos et al., 1988; Heginbotham and Dunwiddie, 1991). However, there are several possible explanations for the prolonged effects of ISO, cAMP analogs, and forskolin. One possibility is that the substrates that are phosphorylated by cAMP-dependent protein kinase are dephosphorylated with a relatively slow time course, or that autophosphorylation of a kinase may lead to its persistent activation. In this situation, it would be the persistence of the phosphorylated substrate that determines the longevity of the response. Alternatively, transiently phosphorylated proteins might in turn induce enduring changes in pyramidal neuron excitability through other mechanisms. A somewhat less likely possibility is that cAMP itself is persistently elevated by treatment with ISO, although there is some precedent for increases of moderate duration in the dentate gyrus (Stanton and Sarvey, 1985b). A possibility that we cannot completely rule out as far as forskolin and the cAMP analogs are concerned is that they may wash out of the tissue very slowly. The PKA inhibitors that would be expected to reverse the effects of these agents had other actions that precluded their use in these experiments. However, the depressant effects of br-cAMP that are mediated via extracellular adenosine receptors were usually reversed within 5 min of washing (Fig. 5*A*), suggesting that this cAMP analog was removed from the extracellular space quite quickly; how-

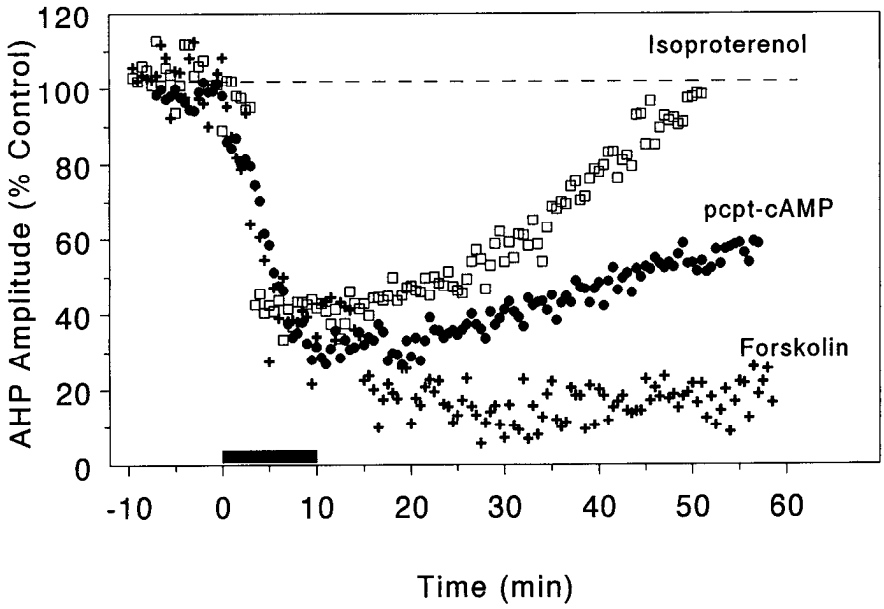

Figure 9. Effect of ISO, pcpt-cAMP, and forskolin on depolarization-induced AHPs. Each set of symbols corresponds to a single pyramidal neuron superfused with ISO (500 nM; *squares*), pcpt-cAMP (100 μM; *circles*), or forskolin (10 μM; *crosses*) during the period indicated by the *bar* along the x-axis. AHPs were evoked by a depolarizing current pulse of 200 msec duration and then were measured at their peak. In these experiments, the effects of these agents upon the resting membrane potential were reversed by injecting a holding current into the neuron to maintain the original resting potential.

ever, these results do not directly address the issue of the rate of washout from the intracellular compartment.

The one observation that does not seem to be in accord with the rest of our findings is that the effects of pcpt-cAMP on extracellular responses were reliably reversed by washing, whereas the effects of the other three cAMP analogs, ISO, and forskolin were persistent. There are at least two possible explanations that might account for this observation. First, the increases in population spikes induced by pcpt-cAMP may result from some other action of this drug unrelated to PKA activation. We have shown that at substantially higher concentrations this analog is a GABA antagonist (Leidenheimer et al., 1990), and a similar type of "nonspecific" action might account for its effects in these studies and their subsequent reversibility. An alternative explanation for the reversibility of the pcpt-cAMP response could be that the persistent effects of mt-cAMP, br-cAMP, dibutyryl-cAMP, and forskolin all reflect poor washout from the tissue and that pcpt-cAMP is the only analog that does wash out with a relatively rapid time course. Given the fact that these are all fairly lipid-soluble agents (so they can readily cross cell membranes), and because hydrophobic drugs such as these often show extremely prolonged washout from brain slices, this latter alternative remains a possibility. If this were the case, then it would be necessary to conclude that the persistence of the response to ISO itself reflects a consistently elevated level of cAMP, such as has been reported in dentate gyrus (Stanton and Sarvey, 1985b), in order to explain why ISO but *not* pcpt-cAMP produced long-term effects.

The results of these experiments, in combination with those of our previous study (Heginbotham and Dunwiddie, 1991), have helped to clarify the relationship between the acute effects of ISO and BAP. Several lines of evidence suggest that they can be dissociated under certain conditions. First, although virtually every slice shows short-term increases in population spike amplitude, not all slices show BAP, and in some slices the decline in the population spike response during washout is approximately the washout time of the chamber (Heginbotham and Dunwiddie, 1991). Second, br-cAMP, and in some cases forskolin, produced an acute depression or even complete loss of the population spike response, yet still induced the long-term effect. Third, prior stimulation with multiple LTP trains prevents BAP but not the short-term response to ISO (M. Taylor and T. V. Dunwiddie, unpublished observations). These results suggest that either there are two independent processes, one transient and one persistent, that can be activated by ISO, or that other unknown factors can influence the longevity of the initial response to ISO in hippocampus. One possibility that we have not fully explored is that there may be a threshold effect in terms of BAP, such that if the initial response is either not large enough or is not of sufficient duration, then the long-term response does not occur. Previous studies in the dentate gyrus have suggested that a different kind of persistent effect, NE-induced long-lasting potentiation (NE-LLP), may depend upon the duration of the NE treatment. Long-lasting effects were not observed at all with transient activation of the locus coeruleus (Harley and Milway, 1986), were seen approximately 20% of the time with 10 μM NE perfusion for 10 min in brain slices (Lacaille and Harley, 1985), and were consistently observed with 30 μM NE for 30 min (Stanton and Sarvey, 1985c). Intracellular studies in the CA1 region have usually reported quite good reversibility of these responses when the period of drug superfusion is transient (Madison and Nicoll, 1986a,b). The mechanism that underlies this type of threshold behavior is unclear but might involve a requirement for a certain level of initial activation to become self-sustaining. The autophosphorylation of a protein kinase is one such mechanism that might result in a persistent activation of kinase activity, provided a certain threshold level of activation is reached.

Although we have shown that BAP, LTP, and NE-LLP in the dentate gyrus clearly represent different forms of plasticity, the relationship between these phenomena remains to be explored. We have distinguished BAP from both LTP and NE-LLP in several ways. First, LTP is clearly a calcium-dependent process (Malenka et al., 1988); we have previously demonstrated that LTP is markedly reduced under low-calcium conditions (Dunwiddie and Lynch, 1979), yet BAP can be induced under conditions where even synaptic transmission cannot be supported. Both LTP (Harris et al., 1984; Herron et al., 1986; Gustafsson

et al., 1987) and NE-LLP (Burgard et al., 1989; Stanton et al., 1989) are sensitive to 2-amino-5-phosphonovaleric acid, whereas BAP is not (Heginbotham and Dunwiddie, 1991). Furthermore, the fact that we have not observed any changes in the EPSP response with BAP, whereas NE-LLP and LTP both increase the amplitude of the EPSP, suggests that it is possible to distinguish BAP clearly from these other forms of plasticity.

Another possible relationship between BAP, NE-LLP, and LTP is suggested by our observation that ISO can induce a prolonged depolarization of pyramidal neurons, similar to what occurs in dentate granule neurons (Stanton et al., 1989). On this basis, we hypothesize that BAP might facilitate the induction of LTP, particularly with near threshold stimuli, because the β-adrenergic receptor–mediated depolarization and the antagonism of AHPs would tend to relieve the voltage-dependent block of NMDA channels. A synergistic effect of NE and high-frequency stimulation has been reported in the CA3 region, where β-adrenergic receptor activation increases both the magnitude and duration of LTP in the mossy fibers (Hopkins and Johnston, 1984). Whether a similar response occurs in the dentate gyrus and in the CA1 region remains to be determined.

In summary, these experiments clearly support the hypothesis that transient activation of β-adrenergic receptors in the CA1 region can induce a long-term increase in the excitability of the pyramidal neurons. Furthermore, this increased excitability can be elicited by a variety of treatments that activate cAMP-dependent protein kinase, suggesting that this is the most likely effector system through which this effect occurs. Finally, the data from intracellular recording studies and the low-calcium bursting experiments demonstrate that these effects are probably postsynaptic in origin and may result from a maintained depolarization of the pyramidal neurons. The functional role played by BAP in normal electrophysiological activity in the hippocampus is unclear. However, a persistent depolarization of the pyramidal neurons and reduction in the AHP would increase the likelihood of triggering LTP, in that it would reduce the voltage-dependent block by magnesium of the NMDA receptor channels, and would increase the chances of initiating this form of lasting plasticity. The moderately long time course of BAP ($t_{1/2} \approx 2$ hr; Heginbotham and Dunwiddie, 1991) would suggest that rather than being a substrate for learning per se, it is more likely that activation of β-adrenergic receptors in the hippocampus initiates a transitory state that lasts somewhere between minutes and hours, during which the likelihood of initiating more enduring forms of plasticity is enhanced.

References

Burgard EC, Decker G, Sarvey JM (1989) NMDA-receptor antagonists block norepinephrine-induced long-lasting potentiation and long-term potentiation in rat dentate gyrus. Brain Res 482:351–355.

Corradetti R, Pugliese AM, Ropert N (1989) The protein kinase C inhibitor 1-(5-isoquinolinesulphonyl)-2-methylpiperazine (H-7) disinhibits CA1 pyramidal cells in rat hippocampal slices. Br J Pharmacol 98:1376–1382.

Dunwiddie TV, Fredholm BB (1989) Adenosine A1 receptors inhibit adenylate cyclase activity and neurotransmitter release and hyperpolarize pyramidal neurons in rat hippocampus. J Pharmacol Exp Ther 249:31–37.

Dunwiddie TV, Hoffer BJ (1980) Adenine nucleotides and synaptic transmission in the *in vitro* rat hippocampus. Br J Pharmacol 69:59–68.

Dunwiddie TV, Lynch GS (1979) The relationship between extracellular calcium concentrations and the induction of hippocampal long-term potentiation. Brain Res 169:103–110.

Freedman R, Hoffer BJ, Woodward DJ, Puro D (1977) Interaction of norepinephrine with cerebellar activity evoked by mossy and climbing fibers. Exp Neurol 55:269–288.

Gustafsson B, Wigstrom H, Abraham WC, Huang YY (1987) Long-term potentiation in the hippocampus using depolarizing current pulses as the conditioning stimulus to single volley synaptic potentials. J Neurosci 7:774–780.

Haas HL (1986) Long-term potentiation and intrinsic disinhibition. In: Learning and memory: mechanisms of information storage in the nervous system (Matthies H, ed), pp 41–49. Oxford: Pergamon.

Haas HL, Konnerth A (1983) Histamine and noradrenaline decrease calcium-activated potassium conductance in hippocampal pyramidal cells. Nature 302:432–434.

Harley CW, Milway JS (1986) Glutamate ejection in the locus coeruleus enhances the perforant path–evoked population spike in the dentate gyrus. Exp Brain Res 63:143–150.

Harris EW, Ganong AH, Cotman CW (1984) Long-term potentiation in the hippocampus involves activation of *N*-methyl-D-aspartate receptors. Brain Res 323:132–137.

Heginbotham LR, Dunwiddie TV (1991) Long term increases in the evoked population spike in the CA1 region of rat hippocampus induced by beta-adrenergic receptor activation. J Neurosci 11:2519–2527.

Herron CE, Lester RA, Coan EJ, Collingridge GL (1986) Frequency-dependent involvement of NMDA receptors in the hippocampus: a novel synaptic mechanism. Nature 322:265–268.

Hoffer BJ, Siggins GR, Bloom FE (1971) Studies on norepinephrine-containing afferents to Purkinje cells of rat cerebellum. II. Sensitivity of Purkinje cells to norepinephrine and related substances administered by microiontophoresis. Brain Res 25:523–534.

Hoffer BJ, Siggins GR, Oliver AP, Bloom FE (1973) Activation of the pathway from locus coeruleus to rat cerebellar Purkinje neurons: pharmacological evidence of noradrenergic central inhibition. J Pharmacol Exp Ther 184:553–569.

Hopkins WF, Johnston D (1984) Frequency-dependent noradrenergic modulation of long-term potentiation in the hippocampus. Science 226:350–352.

Hopkins WF, Johnston D (1988) Noradrenergic enhancement of long-term potentiation at mossy fiber synapses in the hippocampus. J Neurophysiol 59:667–687.

Kasamatsu T, Shirokawa T (1985) Involvement of β-adrenoceptors in the shift of ocular dominance after monocular deprivation. Exp Brain Res 59:507–514.

Kostopoulos G, Psarropoulou C, Haas HL (1988) Membrane properties, response to amines and to tetanic stimulation of hippocampal neurons in the genetically epileptic mutant mouse tottering. Exp Brain Res 72:45–50.

Lacaille JC, Harley CW (1985) The action of norepinephrine in the dentate gyrus: beta-mediated facilitation of evoked potentials *in vitro*. Brain Res 358:210–220.

Leidenheimer NJ, Dunwiddie TV, Hahner LD, Harris RA (1990) Direct effects of second messenger system modulators on the $GABA_A$ receptor complex. Mol Pharmacol 38:823–828.

Madison DV, Nicoll RA (1982) Noradrenaline blocks accommodation of pyramidal cell discharge in the hippocampus. Nature 299:636–638.

Madison DV, Nicoll RA (1986a) Actions of noradrenaline recorded intracellularly in rat hippocampal CA1 pyramidal neurones *in vitro*. J Physiol (Lond) 372:221–244.

Madison DV, Nicoll RA (1986b) Cyclic adenosine 3′,5′-monophosphate mediates β-receptor actions of noradrenaline in rat hippocampal pyramidal cells. J Physiol (Lond) 372:245–259.

Malenka RC, Kauer JA, Zucker RS, Nicoll RA (1988) Postsynaptic calcium is sufficient for potentiation of hippocampal synaptic transmission. Science 242:81–83.

Mueller AL, Hoffer BJ, Dunwiddie TV (1981) Noradrenergic responses in rat hippocampus: evidence for mediation by alpha and beta receptors in the *in vitro* slice. Brain Res 214:113–126.

Muller D, Turnbull J, Baudry M, Lynch GS (1988) Phorbol ester induced synaptic facilitation is different than long-term potentiation. Proc Natl Acad Sci USA 8597:6997–7000.

Pang K, Rose GM (1987) Differential effects of norepinephrine on hippocampal complex-spike and theta neurons. Brain Res 425:146–158.

Pettigrew JD, Kasamatsu T (1978) Local perfusion of noradrenaline maintains visual cortical plasticity. Nature 271:761–763.

Rose GM, Pang KC (1989) Differential effect of norepinephrine upon granule cells and interneurons in the dentate gyrus. Brain Res 488: 353–356.

Schubert P, Lee KS (1986) Non-synaptic modulation of repetitive firing by adenosine is antagonized by 4-aminopyridine in a rat hippocampal slice. Neurosci Lett 67:334–338.

Seamon KB, Padgett W, Daly JW (1981) Forskolin: unique diterpene activation of adenylate cyclase in membrane and intact cells. Proc Natl Acad Sci USA 7863:3363–3371.

Segal M, Bloom FE (1974a) The action of norepinephrine in the rat hippocampus. I. Iontophoretic studies. Brain Res 72:79–97.

Segal M, Bloom FE (1974b) The action of norepinephrine in the rat hippocampus. II. Activation of the input pathway. Brain Res 72:99–114.

Siggins GR, Oliver AP, Hoffer BJ, Bloom FE (1971) Cyclic adenosine monophosphate and norepinephrine: effects on transmembrane properties of cerebellar Purkinje cells. Science 171:192–194.

Stanton PK, Sarvey JM (1985a) Depletion of norepinephrine, but not serotonin, reduces long-term potentiation in the dentate gyrus of rat hippocampal slices. J Neurosci 5:2169–2176.

Stanton PK, Sarvey JM (1985b) The effect of high-frequency electrical stimulation and norepinephrine on cyclic AMP levels in normal versus norepinephrine-depleted rat hippocampal slices. Brain Res 358: 343–348.

Stanton PK, Sarvey JM (1985c) Blockade of norepinephrine-induced long-lasting potentiation in the hippocampal dentate gyrus by an inhibitor of protein synthesis. Brain Res 361:276–283.

Stanton PK, Sarvey JM (1987) Norepinephrine regulates long-term potentiation of both the population spike and dendritic EPSP in hippocampal dentate gyrus. Brain Res Bull 18:115–119.

Stanton PK, Mody I, Heinemann U (1989) A role for *N*-methyl-D-aspartate receptors in norepinephrine-induced long-lasting potentiation in the dentate gyrus. Exp Brain Res 77:517–530.

Woodward DJ, Moises HC, Waterhouse BD, Hoffer BJ, Freedman R (1979) Modulatory actions of norepinephrine in the central nervous system. Fed Proc 38:2109–2116.