Glutamate, the main transmitter in hippocampal neurons, is released by input stimulation of CA1 neurons [1]. Moreover, depending on the stimulation frequency, adenosine 5'-triphosphate (ATP) and adenosine derivatives, common constituents of synaptic vesicles [2], are released as cotransmitters at hippocampal CA1 synapses [3].

In the CA1 area of the hippocampus where adenosine A1 receptors are known to be present at high levels [4], adenosine depresses excitatory postsynaptic potentials (EPSP) and hyperpolarises pyramidal neurons [5, 6] by activating A1 adenosine receptors [7, 8].

Besides its effects at excitatory sites, there is evidence that adenosine attenuates the strength of the inhibitory circuit in CA1 neurons [9–12]. A recent study [13] showed that in CA1 neurons in hippocampal slices the inhibition of excitatory synaptic transmission in response to hyperthermia can be blocked by adenosine A1 receptor antagonists and by adenosine deaminase, suggesting that it is mediated by increased activation of presynaptic A1 receptors. However, the effects of temperature on the inhibitory circuit in CA1 neurons in hippocampal slices have not been extensively characterized, especially in regard to

**Abstract:** We examined the effects of temperature on excitatory synaptic transmission and the recurrent inhibitory loop in CA1 neurons in guinea pig hippocampal slices. Increasing the temperature of the perfusing medium from 30 to 49°C resulted in attenuation of both the amplitude of the synaptically evoked CA1 population spikes and the paired-pulse inhibition (PPI) of the spikes. A bath application of 2 μM picrotoxin, a γ-aminobutyric acid receptor antagonist, did not affect the amplitude of the CA1 population spikes, but it significantly reduced PPI during the early heating phase (30–32°C). In contrast, the application of 1 mM theophylline or 50 μM 8-phenyltheophylline, a selective adenosine A1 receptor antagonist, resulted in significant augmentation of the PPI during the early phase of hyperthermia (30–34°C) and a significant increase in the amplitude of the CA1 population spikes at higher temperatures (34–43°C). These results suggest that increased activation of adenosine A1 receptors in response to a temperature increase depresses not only excitatory synaptic responses, but also the strength of the inhibitory circuit in CA1 neurons. Furthermore, hyperexcitability of CA1 pyramidal neurons was seen in the middle of the heating range (34–38°C), excitatory responses still being present, but the strength of the inhibitory circuit significantly reduced. [Japanese Journal of Physiology, 51, 545–554, 2001]

**Key words:** temperature, hippocampus, adenosine, paired-pulse inhibition, synaptic transmission.
endogenous adenosine levels.

The hippocampal slice preparation is a useful model system for studying the function and modulation of interneuronal loops [14]. By applying paired-pulse stimulation with two selectively located stimulating electrodes (Fig. 1, A–C), an inhibitory feedback loop can be activated [15, 16] and the effects of adenosine on this circuit evaluated [9]. Using this approach, we have characterized the effects of a temperature increase on the population spikes evoked by paired-pulse stimulation and examined the extent to which this can be attributed to changes in endogenous adenosine levels. In the present study, using (−)-N\(^6\)-\(N\(^2\)-2-phenylisopropyl)-adenosine (L-PIA), a selective adenosine A1 receptor agonist [17, 18], and 8-phenyltheophylline (8-PT), a selective adenosine A1 receptor antagonist [17], we have shown that adenosine A1 receptor activation is involved in the effects of a temperature increase on an inhibitory or an excitatory synaptic transmission at CA1 synapses in the guinea pig hippocampus.

MATERIALS AND METHODS

The animals used were maintained and handled following the guidelines of the Animal Care and Use Committee of Yamagata University School of Medicine. Sixty-one hippocampal slices (400\(\mu\)m), prepared from 22 adult male Hartley guinea pigs (200–350 g), were preincubated for at least 1 h at 30–32°C in a standard medium consisting (in mM) of NaCl 124, KCl 5.0, NaH\(_2\)PO\(_4\) 1.25, MgSO\(_4\) 2.0, CaCl\(_2\) 2.0, NaHCO\(_3\) 22.0, and glucose 10.0, pH 7.38–7.45, equilibrated with 95% O\(_2\)–5% CO\(_2\). A slice was transferred to and fixed on a mesh in a recording chamber to allow perfusion (2–3 ml/min) of both sides of the slice with the standard medium at 30°C.

As shown in Fig. 1A, two bipolar stimulating electrodes were used, one (S1) being inserted in the stratum radiatum to stimulate the input pathways to the CA1 neurons orthodromically and the other (S2) in the alveus to stimulate the axons antidromically. A glass pipette filled with 2 M NaCl was placed in the CA1 pyramidal cell layer to record the orthodromic or antidromic population spike (PS). Stimulation was applied either to S1 alone (single-pulse stimulation) or initially to S2, then to S1 10 ms later (paired-pulse stimulation). As shown in Fig. 1B, paired-pulse stimulation induced paired-pulse inhibition (PPI) of the PS elicited by the second of the paired stimuli, the amplitude of the PS (elicited by a single orthodromic stimulation (X) and that elicited by the second of the paired stimuli (Y) were measured during heating. (C) Illustration of the excitatory and inhibitory circuitries of CA1 neurons. Stimulation applied to S1 activates excitatory synaptic transmission between Schaffer collaterals (Sch) and CA1 pyramidal cells (P). Stimulation of the alveus (S2) antidromically activates the pyramidal cells (P) and the pyramidal axon collaterals that innervate the inhibitory interneurons (P).
To check the stability of the responses, the test stimulus was repeated every 20 s at 30°C and control responses were recorded for a minimum of 30 min. After confirming that the variance in amplitude of the antidromic and orthodromic PS was less than 0.2 mV in 10 min, the temperature of the perfusing medium was increased from 30°C to various temperatures (45–48°C) at a rate of 0.5°C/min, using a small heater element submerged in the perfusate in the recording chamber. During the entire heating process, single-pulse stimulation and paired-pulse stimulation were delivered alternately at 5 s intervals to follow rapid changes in responses. The temperature of the perfusion medium was monitored continuously by using a needle-type thermometer placed near the CA1 region of the slice or inserted into the dentate gyrus. In experiments, the mean value for the PS in response to S1 stimulation (X value, Fig. 1B) in the 10 min immediately before heating (30°C) was defined as the 100% level, and changes in responses during heating were expressed as the mean ± SEM (%) of this control level. The PS was considered to be abolished when it fell to 10% of the control level.

The amplitude of the PS elicited by the second of the paired stimuli (Y in Fig. 1B2) was also measured during heating. The PPI was expressed as the percentage amplitude response ([Y/X]×100). The percentage changes in the orthodromic PS amplitude and in the PPI during heating were compared in control slices and in slices perfused continuously with picrotoxin (PTX, 2 µM), theophylline (1 mM), 8-PT (50 µM), or L-PIA (5 µM) (all from Research Biochemical Inc., Natic, MA, USA). The results were analyzed for significance (p<0.05 or 0.01) by using a two-tailed Student’s t-test.

During heating from 30 to 40°C, multiple PSs were frequently elicited by a single orthodromic stimulation. The 30–40°C temperature range was divided into five 2°C intervals, and the number of times multiple population spikes began to appear in response to a single orthodromic stimulus in each range was noted (Table 1). A frequency analysis was performed by using Fisher’s exact test to evaluate whether the application of test reagents correlated with the appearance of repetitive orthodromic PSs during heating.

### RESULTS

**Temperature-induced changes in the PS amplitude in response to a single stimulus or in the PPI in response to a paired stimulus**

When the perfusate temperature of a single slice was gradually increased from 30 to 45–48°C and the responses measured at the indicated temperatures, changes occurred in the amplitude of the PS elicited in response to a single orthodromic stimulation or a paired-pulse stimulation. Typical examples are shown in Fig. 2A. At 30–34°C, the amplitude of the PS elicited by the second of the paired stimuli was smaller than that elicited by single orthodromic stimulation. When the temperature reached 35°C, the amplitude of the orthodromic PS began to decrease, and multiple PSs were frequently elicited. Furthermore, the PPI decreased as the temperature increased, with the result that at 37°C the amplitude of the PS elicited by the second of the paired stimuli became equal to that elicited by a single orthodromic stimulation. When the temperature reached 41°C, the orthodromic PS was abolished, but the antidromic PS was maintained.

Figure 2B shows the temperature dependency of the average amplitude of the orthodromic PS for 25 slices. The amplitude of the orthodromic PS, which decreased continuously during heating, was significantly reduced above 34°C, showing a profound reduction in action potential formation in the pyramidal cells. As the temperature was increased further, the

### Table 1. Temperature at which multiple population spikes appeared in the orthodromic responses.

<table>
<thead>
<tr>
<th>Temperature (°C)</th>
<th>30–31.9</th>
<th>32–33.9</th>
<th>34–35.9</th>
<th>36–37.9</th>
<th>38–39.9</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>9/25 (36.0%)</td>
<td>0</td>
<td>1</td>
<td>4</td>
<td>4</td>
</tr>
<tr>
<td>PTX</td>
<td>7/11 (63.6%)</td>
<td>3</td>
<td>3</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>Theophylline</td>
<td>10/25 (40.0%)</td>
<td>0</td>
<td>1</td>
<td>5</td>
<td>4</td>
</tr>
<tr>
<td>L-PIA</td>
<td>4/10 (40.0%)</td>
<td>2</td>
<td>1</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>8-PT</td>
<td>3/6 (50.0%)</td>
<td>0</td>
<td>0</td>
<td>1</td>
<td>2</td>
</tr>
</tbody>
</table>

The orthodromic response was elicited in the absence (control) or presence of PTX (picrotoxin, 2 µM) or theophylline (1 mM). n, total number of slices in which multiple population spikes were elicited in the orthodromic responses; N, total number of slices tested.
orthodromic PS was abolished at 41.1 ± 0.4°C (n=25), and the antidromic PS disappeared at the significantly higher temperature of 46.1 ± 0.8°C (n=25, p<0.01), indicating the greater susceptibility of the orthodromic PS to hyperthermic insult.

Figure 2C shows the temperature dependency of the average percentage amplitude response in 25 slices, showing a decrease in PPI during heating. The average percentage amplitude was 71.9 ± 4.5% at 30°C and 85.3 ± 3.3% at 34°C (n=25), and at 35 and 37°C it was 92.5 ± 5.2 and 98.4 ± 5.4%, respectively, both significantly higher than that at 30°C, indicating a profound reduction in the influence of the interneuronal loops on the PS as the temperature increased.

Nine of 25 slices showed more than two repetitive PSs following a single orthodromic stimulation during heating (Table 1), but no multiple PSs were seen in the antidromic responses in any slice. The mean threshold temperature at which more than two repetitive PSs first appeared following a single orthodromic stimulation was 35.4 ± 0.5°C (n=9). Thus it is possible that GABA receptor-mediated synaptic inhibition at the soma or the initial segment of the axon of CA1 pyramidal neurons, which normally follows excitation of CA1 pyramidal neurons, might be reduced by the temperature increase and thus allow multiple spike discharge of CA1 pyramidal neurons following single orthodromic stimulation.

PTX modulation of temperature-induced changes in the PPI

The effects of temperature on the amplitude of the PS in response to single orthodromic stimulation and on the PPI in response to paired-pulse stimulation were measured in the presence of 2 μM PTX, which blocks GABA receptor-mediated feedback inhibition in CA1 pyramidal cells. As shown in the typical examples in Fig. 3A, multiple PSs were frequently elicited in the presence of PTX in response to a single orthodromic stimulation, but no PPI was seen at any temperature.

The temperature dependency of the average amplitude of the orthodromic PS in response to single pulse stimulation was compared in control slices (n=25) and slices perfused with 2 μM PTX (n=11); Fig. 3B shows that this was unaffected by PTX. In contrast, the temperature dependency of the average change in PPI (average percentage amplitude) in control slices (n=25) and in slices perfused with 2 μM PTX (n=11) (Fig. 3C) shows that the average percentage amplitude was significantly higher in the presence of PTX than in controls at 30°C (99.8 ± 5.1%) and at 32°C (103.5 ± 8.2%) (**p<0.01 or *p<0.05), indicating that the influence of the interneuronal loops on the PS was abol-
ished in 2 μM PTX in the lower heating range.

Seven of 11 slices showed more than two repetitive PSs following a single orthodromic stimulation during heating (Table 1), but multiple PSs were not seen in the antidromic responses in any slice. Fisher’s exact test showed no significant difference in the frequency of multiple PSs following a single orthodromic stimulation in the absence or presence of 2 μM PTX during heating (9/25 slices compared with 7/11, *p* = 0.12). However, the mean threshold temperature at which more than two repetitive PSs was evoked following a single orthodromic stimulation in these PTX-treated slices was 33.0 ± 0.8°C (*n* = 7), significantly (*p* < 0.05) lower than that in the absence of PTX. Thus it is possible that a reduction of GABA-mediated synaptic inhibition in CA1 pyramidal neurons was enhanced in the presence of 2 μM PTX, allowing multiple spike discharge of CA1 pyramidal cells at a significantly lower temperature than in the standard solution.

**Theophylline modulation of temperature-induced changes in the orthodromic PS and the PPI**

The effects of temperature on the PS amplitude in response to a single orthodromic stimulation and on the PPI were measured in the presence of 1 mM theophylline that blocks the inhibitory effects of adenosine on synaptic transmission of the excitatory or inhibitory circuit in the CA1 region (Fig. 1C). Figure 4A shows a typical example in which the orthodromic PS showed an increased amplitude and a decreased latency up to 35°C, but as the temperature was raised above this level, a decrease in amplitude, together with repetitive PSs, was seen. In this example, the antidromic PS showed a decreased amplitude during heating to 43°C, but both orthodromic and antidromic PSs were still seen at temperatures of 43.0°C. An increase in PPI was seen during the early heating phase (30–34°C).

We then compared the temperature-induced changes in the orthodromic PS amplitude and the PPI in control slices (*n* = 25) and slices perfused with 1 mM theophylline (*n* = 25). As shown in Fig. 4B, in the range of 35–41°C the percentage amplitudes of the PS elicited in 1 mM theophylline were significantly (*p* < 0.05 or ***p* < 0.01) higher than in the absence of 1 mM theophylline. When the temperature was further increased in the presence of 1 mM theophylline, the orthodromic PS was abolished at 43.3 ± 0.3°C (*n* = 25), and the antidromic PS was abolished at 46.5 ± 0.3°C (*n* = 25, *p* < 0.01). The former value is significantly (***p* < 0.01) higher than the values seen in the control slices, but the latter value does not differ significantly from control values. These results suggest that 1 mM theophylline might antagonize the temperature-induced inhibitory effects on synaptically evoked CA1 population spikes and maintain the capacity for action potential formation in pyramidal cells at a temperature at which the orthodromic PS was abolished in the standard solution.

Figure 4C shows the temperature-dependency of the PPI in the presence and absence of 1 mM theophylline. The average percentage amplitude in the presence of 1 mM theophylline was 59.3 ± 3.7% at 30°C, 61.3 ±
5.7% at 32°C, and 72.4±5.2% at 34°C, significantly lower (*p<0.01 or **p<0.05) than in the absence of theophylline. This indicates that the influence of the interneuronal loops on the PS was increased at 30–34°C in 1 mM theophylline. Thus it is possible that theophylline might also antagonize the temperature-dependent inhibitory effects on synaptic transmission of the inhibitory circuit in the CA1 region.

Ten of 25 slices perfused with 1 mM theophylline showed more than two repetitive PSs following a single orthodromic stimulation during heating (Table 1), but no multiple PSs were seen in the antidromic responses in any slice. Fisher’s exact test showed no difference in the frequency of multiple PSs in response to orthodromic stimulation in the presence or absence of 1 mM theophylline during heating (9/25 slices compared with 10/25, p>0.5). However, the mean threshold temperature at which more than two repetitive PSs were evoked following a single orthodromic stimulation was 36.9±0.5°C in the theophylline-treated slices (n=10), significantly (p<0.05) higher than in the control slices. These results suggest that GABA-mediated synaptic inhibition in CA1 pyramidal neurons is increased in the presence of theophylline and results in a multiple spike discharge of CA1 population spikes not being seen until a higher temperature than in the standard solution.

### Modulation of temperature-induced changes in the orthodromic PS and the PPI by an antagonist and an agonist of adenosine A1 receptors

Since theophylline is considered an adenosine receptor antagonist [17, 19], the results of the present study suggest that 1 mM theophylline might antagonize the temperature-induced inhibitory effects of adenosine on the excitatory or the inhibitory synaptic transmission in the CA1 region. To verify the temperature-dependent inhibitory effects of adenosine on the excitatory or the inhibitory circuit in the CA1 region, the effects of temperature on the amplitude of the PS in response to single orthodromic stimulation and on the PPI in response to paired-pulse stimulation were measured in the presence of 5 μM L-PIA, an adenosine A1 receptor agonist, or 50 μM 8-PT, an adenosine A1 receptor antagonist.

We first compared the temperature-induced changes in the orthodromic PS amplitude and the PPI in control slices (n=25) and slices perfused with 5 μM L-PIA (n=10). The upper panel in Fig. 5A shows a typical example recorded in the presence of 5 μM L-PIA (L-PIA), in which the orthodromic PS showed a decreased amplitude up to a temperature of 35°C, but no PPI was seen at 30 and 35°C. As shown in Fig. 5B, in the range of 37–41°C the percentage amplitudes of the PS elicited was significantly (*p<0.05 or **p<0.01) smaller in 5 μM L-PIA than in the absence of these reagents. The orthodromic PS was abolished at 39.3±0.4°C, and the antidromic was abolished at
Effects of Temperature on Inhibitory Circuit

Fig. 5. Effects of a agonist or an antagonist of adenosine A1 receptors on temperature-induced responses. Hippocampal slices were superfused with 5 μM L-PIA or 50 μM 8-PT during heating. (A) PSs elicited in L-PIA (upper) and in 8-PT (lower), in response to a single-pulse stimulation (left) or a paired-pulse stimulation (right) recorded at the indicated temperatures. (B) Change in average PS amplitude elicited by a single orthodromic stimulation in the presence (L-PIA, □, n=10 or 8-PT, △, n=6) or absence (control, ○, n=25) of 5 μM L-PIA or 50 μM 8-PT during heating. * and ** denote significant differences in the average PS amplitude (*p<0.05 or **p<0.01) in control and theophylline-treated slices. (C) Change in PPI in the presence (L-PIA, □, n=10 or 8-PT, △, n=6) or absence (control, ○, n=25) of 5 μM L-PIA or 50 μM 8-PT during heating. * and ** denote significant differences (*p<0.05 or **p<0.01) between control and PIA-treated slices or 8-PT treated slices.

46.9±0.3°C in 5 μM L-PIA (n=10). The former value is significantly (p<0.01) lower than the values seen in the control slices, but the latter value does not differ significantly from control values. Figure 5C shows that the average percentage amplitude was significantly higher in the presence of L-PIA than in controls at 30°C (92.3±2.7%) and at 32°C (93.1±4.2%) (**p<0.01 or *p<0.05), indicating that the influence of the interneuronal loops on the PS was abolished in 5 μM L-PIA in the lower heating range. These results indicate that extracellular adenosine acting via A1 receptors enhanced the temperature-dependent inhibitory effects on synaptic transmission of the excitatory and inhibitory circuits in the CA1 region.

Four of 10 slices perfused with 5 μM L-PIA showed more than two repetitive PSs following a single orthodromic stimulation during heating (Table 1), but no multiple PSs were seen in the antidromic responses in any slice. Fisher's exact test showed no difference in the frequency of multiple PSs in response to orthodromic stimulation in the presence or absence of 5 μM L-PIA during heating (4/10 slices compared with 9/25, p>0.5). However, the mean threshold temperature at which more than two repetitive PSs were evoked following a single orthodromic stimulation was 32.5±0.6°C in the PIA-treated slices (n=4), significantly (p<0.01) lower than in the control slices. These results indicate that extracellular adenosine acting via A1 receptors decreased GABA-mediated synaptic inhibition in CA1 pyramidal neurons and induced multiple spike discharge of CA1 population spikes being seen at a lower temperature than in the standard solution.

We then compared the temperature-induced changes in the orthodromic PS amplitude and the PPI in control slices (n=25) and slices perfused with 50 μM 8-PT (n=6). The lower panel in Fig. 5A shows a typical example recorded in the presence of 50 μM 8-PT (8-PT), in which PPI clearly seen at 30°C was abolished up to a temperature of 41°C. As shown in Fig. 5B, in the range of 39–41°C, the percentage amplitudes of the PS elicited was significantly (**p<0.01) larger in 50 μM 8-PT than in the absence of these reagents. The orthodromic PS was abolished at 44.6±0.9°C (n=6) in 50 μM 8-PT, and the antidromic was abolished at 46.5±0.4°C in 50 μM 8-PT (n=6). The former value is significantly (p<0.01) higher than the values seen in the control slices, but the latter value does not differ significantly from control values. These results thus indicate that the temperature-induced inhibitory effects of adenosine on synaptically evoked CA1 population spikes are mediated by extracellular adenosine via activation of A1 receptors.

The temperature dependency of the average change in PPI was then compared in control slices (n=25) and slices perfused with 50 μM 8-PT (n=6). Figure 5C shows that the average percentage amplitude was significantly lower (p<0.05) in the presence of 8-CPT at 32°C (70.1±4.3%), indicating that the influence of the interneuronal loops on the PS was enhanced in
50 μM 8-PT in the lower heating range. Thus we concluded that the temperature-dependent modulation of an inhibitory circuit in hippocampal CA1 region is mediated by extracellular adenosine via the activation of A1 receptors.

Three of 6 slices perfused with 50 μM 8-PT showed more than two repetitive PSs following a single orthodromic stimulation during heating (Table 1), but no multiple PSs were seen in the antidromic responses in any slice. Fisher’s exact test showed no difference in the frequency of multiple PSs in response to orthodromic stimulation in the presence or absence of 50 μM 8-PT during heating (3/6 slices compared with 9/25, p=0.43). The mean threshold temperature at which more than two repetitive PSs was evoked following a single orthodromic stimulation was 36.5±0.8°C in the 8-PT–treated slices (n=3). This value, however, shows no statistical difference from the value in the control slices.

**DISCUSSION**

In this study, the amplitude of the PS elicited by a single orthodromic stimulation was measured during heating to quantify the change in pyramidal cell excitation evoked by excitatory glutamatergic synaptic input to CA1 neurons. Moreover, the change in the PPI was measured during heating to assess the effect of the temperature increase on the strength of the interneuronal loops.

Orthodromic responses are reported to be more susceptible than antidromic responses to oxygen deprivation [20–22] or glucose levels [22, 23]. The same trend in neuronal properties was seen in the present study, with antidromic responses being more resistant than orthodromic responses to high temperatures in terms of the disappearance of electrical activities (Figs. 2A, 3A, and 4A). Since an orthodromic PS reflects synaptic transmission and an antidromic PS reflects axonal and somal functions, these results indicate that in CA1 neurons synaptic transmission is more susceptible than axonal or somal function to hyperthermia.

The decrease in and disappearance of the synaptic responses in CA1 neurons can be partially explained by the decreased input resistance of the postsynaptic cell membrane as a result of the temperature increase [24]. It can also be explained by the hypoxic conditions to which the neuronal tissues were exposed during hyperthermia [25]. Since the increased oxygen consumption and rate of energy use are dependent on the temperature increase, the partial pressure of oxygen in the tissue falls dramatically during hyperthermia [26, 27]. Furthermore, changes in the extracellular concentration of adenosine could also contribute to changes in electrical activity during hyperthermia; this might explain the decrease in amplitude of the orthodromic PS during hyperthermia and the disappearance of the transsynaptic responses after exposure to high temperatures, since the extracellular adenosine concentration in brain tissue is increased by hyperthermia [28] and/or hypoxia [29].

In the CA1 region of the hippocampus, the activation of A1 receptors has a tonic influence on electrophysiological activity in the CA1 region, in which adenosine acts primarily on presynaptic A1 receptors to inhibit excitatory synaptic transmission [8]. Recently, Masino and Dunwiddie [13] demonstrated that in CA1 neurons in hippocampal slices, the inhibition of excitatory synaptic transmission in response to hyperthermia can be blocked by 200 μM theophylline or an adenosine A1 receptor antagonist. The results of the present study also showed that an application of 1 mM theophylline or 50 μM 8-PT, an A1 receptor antagonist, significantly reduced the effects of a temperature increase on the amplitude of synthetically evoked CA1 population spikes at 34–41°C (Figs. 4B and 5B), but an A1 agonist (L-PIA) significantly enhanced them at 37–41°C (Fig. 5B). Considering the binding thermodynamics of adenosine A1 receptors, for which the agonist affinity increases with increased temperature [30], it is possible that the temperature-induced increased activation of adenosine A1 receptors reduces excitatory synaptic inputs to CA1 pyramidal cells.

The results shown in Fig. 2C demonstrate that increasing the temperature of hippocampal slices resulted in a profound decrease in the PPI, indicating that the temperature increase also reduced tonic inhibition to CA1 neurons. Furthermore, the application of 2 μM PTX had no effect on the temperature-induced change in PS amplitude, but it significantly reduced the temperature-induced change in the PPI in the low part of the temperature range (Fig. 3, B and C). These results indicate that 2 μM PTX abolished the influence of the inhibitory interneuronal loops on the PS over the whole heating range without affecting the change in excitatory synaptic responses. Thus it seems that the temperature increase attenuates the strength of the inhibitory circuit independently of the reduction in excitatory synaptic inputs to CA1 pyramidal neurons.

As shown in Fig. 5A, synaptic transmissions in the CA1 inhibitory circuit is composed of excitatory synaptic transmission from pyramidal cell axons to...
the interneurons and inhibitory synaptic transmission from interneuron axons to the pyramidal cells [14]. Previous studies have shown that adenosine also depresses inhibitory postsynaptic potentials (IPSPs) in the CA1 region [6, 9], and these potentials are usually evoked disynaptically. Since the activation of adenosine A1 receptors does not directly modulate GABA release in the CA1 region [10, 31], it is possible that adenosine depresses disynaptic IPSPs by reducing excitatory synaptic transmission from pyramidal cell axons to the interneurons. Since the PPI during the early phase of hyperthermia was significantly enhanced by theophylline or 8-PT, both an adenosine receptor antagonist (Figs. 4C and 5C), but was significantly attenuated by L-PIA, an adenosine A1 receptor agonist, this suggests that an increased activation of adenosine A1 receptors in response to hyperthermia depresses synaptic inputs to the inhibitory interneurons and attenuates the strength of the inhibitory circuit of the CA1 region.

In Fig. 6, the role of adenosine is shown schematically as an inhibitory modulator of synaptic transmission at synapses of the CA1 excitatory and inhibitory circuits. At the control temperature (30°C), adenosine moderately affects synaptic transmission at the synapses between Schaffer collaterals and CA1 pyramidal cells and between the axons of CA1 pyramidal cells and inhibitory interneurons (Fig. 6A). At a higher temperature (above 37°C), excitatory synaptic transmission is reduced by the increased activation of adenosine A1 receptors at these synapses, and consequently, GABA release from the synapses of the inhibitory interneurons might be reduced because of attenuation of the strength of the disynaptic inhibitory circuit (Fig. 6B).

Because repetitive responses were seen in orthodromic PS at a significantly low temperature when the PPI was attenuated by PTX (Fig. 3C and Table 1) or L-PIA (Fig. 5C and Table 1), and at a significantly high temperature when the PPI was enhanced by theophylline (Fig. 4 and Table 1), the reduction in the strength of the inhibitory circuit may play a critical role in the development of pyramidal cell hyperexcitability during hyperthermia. Moreover, the development of CA1 pyramidal cell hyperexcitability following hyperthermia or mild hypoxia [20, 21] seems to increase glutamate release from the synaptic terminals and may contribute to the rise in the extracellular glutamate concentration of these neurons in response to hyperthermia [32, 33] or hypoxia [34].

In conclusion, in this study we have demonstrated that an increase in temperature causes synaptic depression mediated by endogenous adenosine at input synapses to CA1 pyramidal cells and at output synapses from CA1 pyramidal cells to interneurons. Repetitive responses in the orthodromic PS, possibly a result of hyperexcitability of CA1 pyramidal cells, developed during the middle range of hyperthermia (34–38°C), during which the outputs from CA1 pyramidal neurons were depressed by endogenous adenosine and the strength of the inhibitory circuit reduced, and inputs to these cells were maintained. In this regard, an increase in the temperature of hippocampal slices represents a physiological manipulation that markedly affects excitatory synaptic transmission and the inhibitory circuit via endogenous levels of adenosine.
REFERENCES