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Research report

A role for adenosine A_2 receptors in the induction of long-term potentiation in the CA1 region of rat hippocampus

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Abstract

Although reductions in neurotransmission have been reported in response to agonist-mediated adenosine A_1 receptor activation, the implications of A_2 receptor activation on synaptic transmission have not been well explored. We examined the role adenosine A_2 receptors play in the efficacy of neurotransmission between the Schaffer collateral-CA1 pathway in the rat transverse hippocampal slice. A_2 receptor blockade in the presence of complete A_1 receptor inhibition led to a reversible reduction of the field excitatory post-synaptic potential (EPSP) slope in response to low-frequency test pulses (0.033 Hz) indicating that A_2 receptors can enhance synaptic transmission. A_2 receptor blockade by the A_2 antagonist, DMPX (3,7-dimethyl-1-propargylxanthine) prevented the induction of tetanus-induced long-term potentiation (LTP) of the EPSP. In contrast, no such effect on LTP induction was observed during A_1 receptor blockade. We also examined the effects of DMPX on the induction of LTP during continued A_1 receptor blockade with CPT. Under this condition, LTP was significantly reduced when compared to LTP induced in the presence of CPT alone. A similar result was found using the highly polar A_2 antagonist 8-SPT (8-(*p*-sulfophenyl)theophylline) suggesting that the effects of DMPX on LTP were not due to a direct action on an intracellular intermediate. DMPX had no effect on LTP expression if applied 45 min following the tetanus indicating that A_2 receptors play no significant role in the maintenance phase of LTP. Selective A_{2a} receptor activation did not alter the field EPSP. Similarly, selective blockade of the A_{2a} receptor did not interfere with tetanus-induced LTP. Increases in neuronal firing rates can result in elevations in the concentration of extracellular adenosine. Together, these results suggest that the A_2 receptors may play an important role in the induction although not the maintenance of hippocampal LTP and that the effect is likely to be mediated by the A_{2b} receptor.

Keywords: Long-term potentiation; Purinoceptor; Adenosine A_{2b} receptor; Synaptic transmission; Excitatory post-synaptic potential

1. Introduction

Both exogenously applied adenosine as well as more selective A_1 receptor agonists have been shown to diminish the efficacy of synaptic neurotransmission [7,8,18,20]. Because the level of extracellular adenosine in the brain is directly related to levels of neuronal activity, these results have suggested that one role of adenosine may be to down-regulate intercellular communication under conditions of increased neurotransmitter release such as during high firing rates. However, more recent evidence has suggested that activation of receptors other than the A_1 subtype may actually enhance neurotransmission. Adenosine has been shown to produce dose-dependent inhibitory and excitatory effects on neurotransmission in the hippocampus but only excitatory effects in the superior colliculus [15–17] in a cAMP-dependent manner consistent with an A_2 receptor-mediated effect. Furthermore, a role for the adenosine A_2 receptor subtype in hippocampal LTP has been previously proposed [10,22].

Recently, activation of an A_2 receptor in acutely isolated CA3 hippocampal pyramidal neurons has been shown to augment inward Ca current due, at least in part, to potentiation of the ω-agatoxin-IV-A-sensitive P-type Ca channel [14]. Although the relevance of this result to pre- or postsynaptic activity is unknown, it suggests that increased neuronal excitability can result from A_2 receptor activation with possible consequences to synaptic transmission. Therefore, we sought to investigate whether A_2

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receptor activation could play an opposite role to the A_1 receptor; that is, in increasing the efficacy of synaptic transmission. Currently the A_2 receptor has been divided into two subclasses [4,23], namely, the A_{2a} and A_{2b} receptors. Hence, we also sought to determine the specific subclass of A_2 receptors involved.

We studied the effects of selective adenosine receptor agonists and antagonists on extracellularly recorded excitatory post-synaptic potentials (EPSPs) in the CA1 region in response to stimulation of the Schaffer collateral pathway. This region readily demonstrates long-term potentiation (LTP) of synaptic transmission following brief tetanic stimulation. Our results indicate that A_2 receptors are involved in both normal synaptic transmission and in the induction of LTP. Furthermore, because selective A_{2a} receptor activation did not augment field EPSP nor did blockade of these receptors prevent LTP, our results suggest that this effect is mediated in these synapses by A_{2b} receptor activation. The fact that extracellular adenosine levels are modulated by cellular activity throughout the nervous system and that our results indicate a significant role for adenosine receptors in both up- and down-regulation of synaptic transmission suggests that an important and complex role exists for this purine receptor in neurotransmission in the brain.

2. Materials and methods

Hippocampal slices were obtained from 22–35-day-old Sprague–Dawley rats (Harlan Sprague Dawley, Indiannapolis, IN). Animals were anesthetized with isoflurane by inhalation then decapitated. The brain was rapidly excised, hemisected at the interhemispheric fissure, and placed in cold (4°C) artificial cerebrospinal fluid (aCSF) containing (in mM): ωNaCl 124, $NaHCO_3$ 24, D-glucose 10, $MgSO_4$ 1.3, NaH_2PO_4 1.25, KCl 3, $CaCl_2$ 2.4 and gassed with 95% O_2/5% CO_2. Both hippocampi were dissected free and 350 μm transverse slices were cut using a vertical tissue chopper (Stoelting). Slices were stored in the bubbled aCSF at room temperature (approx. 22°C) and transferred as needed to a submersion chamber maintained at 30°C.

Extracellular recordings were made using glass microelectrodes (2–4 MΩ) pulled from borosilicate glass capillaries and filled with NaCl (2 M). Recording electrodes for field EPSPs were placed in stratum radiatum of the CA1 region. Orthodromic stimulation was provided by placement in stratum radiatum near the CA3/CA1 border of a twisted teflon-coated platinum bipolar electrode. Test pulses were delivered at 0.033 Hz. Tetanic stimulation consisted of a 1 s train at 100 Hz delivered at the monitoring intensity. Constant current stimulation (100–400 μA) pulses were delivered by a current stimulus isolator. Stimulus-response curves were constructed by varying pulse width (50–250 μs) and the test stimulus duration for each slice experiment was selected as that required to achieve approx. 50% of the maximum EPSP slope response (usually 60–90 μs). The test duration was not changed within an experiment. Recording signals were amplified using a DAM80 amplifier with active headstage (World Precision Instruments, Sarasota, FL), filtered between 0.1 Hz and 3 kHz, and sampled at 10 kHz by a Digidata 1200 (Axon Instruments, Foster City, CA). Experiments were con-

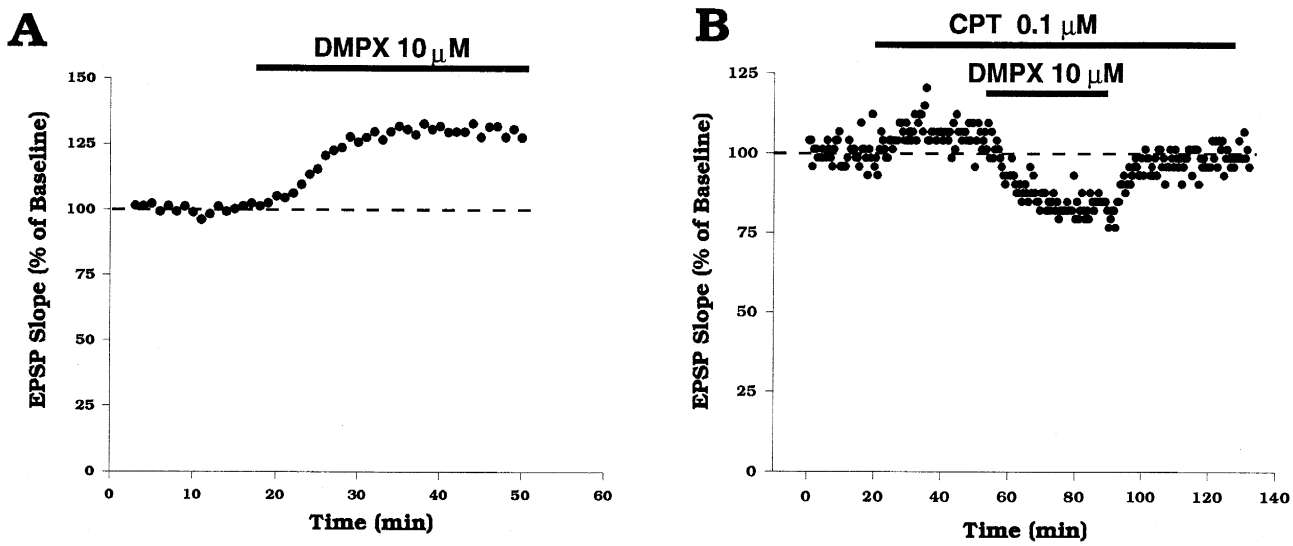

Fig. 1. Blockade of A_2 receptors decreases the field EPSP during low-frequency test pulses. Field EPSP recordings were made in stratum radiatum of the CA1 region. Orthodromic stimulation (0.033 Hz) was delivered via a platinum bipolar electrode placed in the Schaffer collateral pathway. A: DMPX alone increased the field EPSP, presumably due to blockade of adenosine A_1 receptors (see Section 3). B: using a protocol designed to prevent any effects of DMPX on A_1 receptors, the A_1 blocker, CPT, was added causing an increase in the EPSP. However, addition of DMPX resulted in a net decrease in the EPSP below the level observed with CPT alone. DMPX in the presence of CPT resulted in an average reduction of the field EPSP during low frequency test pulses of 8.1 ± 4.9% (mean ± S.E.M.) ($n = 8$).

trolled and analyzed using the Axobasic software (Axon Instr.). The maximum slope of the initial negative deflection for each extracellular field measurement was used to quantify each dendritic EPSP. Adenosine receptor pharmacological agents [CPT, DMPX, CGS 21680, and 8,3-CSC (8-(3-chlorostyryl)caffeine)] were purchased from Research Biochemicals International (Natick, MA). CPT and DMPX were dissolved in aCSF; CGS21680 and 8,3-CSC were dissolved in DMSO. Final concentrations of DMSO never exceeded 0.5%. No effect of this concentration of DMSO on synaptic transmission was observed.

3. Results

3.1. A_2 receptors are involved in normal synaptic transmission

The effects of bath application of the A_2 antagonist, DMPX, on neurotransmission were studied in stratum radiatum of CA1 following orthodromic stimulation of the Schaffer collateral pathway. Although DMPX is a commonly used A_2 antagonist, it does possess a measurable affinity for the A_1 receptor ($K_{d\text{-}A1} = 45\ \mu M$, $K_{d\text{-}A2} = 11\ \mu M$; [23]) hence exposure to DMPX alone (20 μM) can result in reversible increases in the EPSP (Fig. 1A) (130.3 $\pm$ 8%; $n = 6$) qualitatively similar to that seen with A_1 receptor blockade by the selective blocker CPT (198.5 $\pm$ 7.8%; $n = 11$) (mean $\pm$ S.E.M.). Therefore, the effect of A_2 blockade by DMPX on normal synaptic transmission was explored in the presence of A_1 receptor antagonism.

Blockade of the A_1 receptor has been previously shown to augment synaptic transmission presumably through removal of the inhibitory effects of A_1 receptor activation [6]. However, in the presence of the A_1 antagonist, CPT (0.1 μM) sufficient to block all A_1 receptors, exposure to DMPX resulted in a decrease in the EPSP (Fig. 1B) by an average of 8.1 $\pm$ 4.9% ($n = 8$) from that of the level in CPT alone ($P < 0.02$). This result suggests that A_2 receptor activation exerts either an increase in cellular excitability consistent with a population spike enhancement previously reported following A_2 receptor activation in rat hippocampus [21] or an excitatory effect on transmission in these synapses. Because our measurement of field EPSP is much more sensitive to synaptic events, these data would more fully support modification by A_2 receptors of synaptic transmission than of changes to cellular excitability.

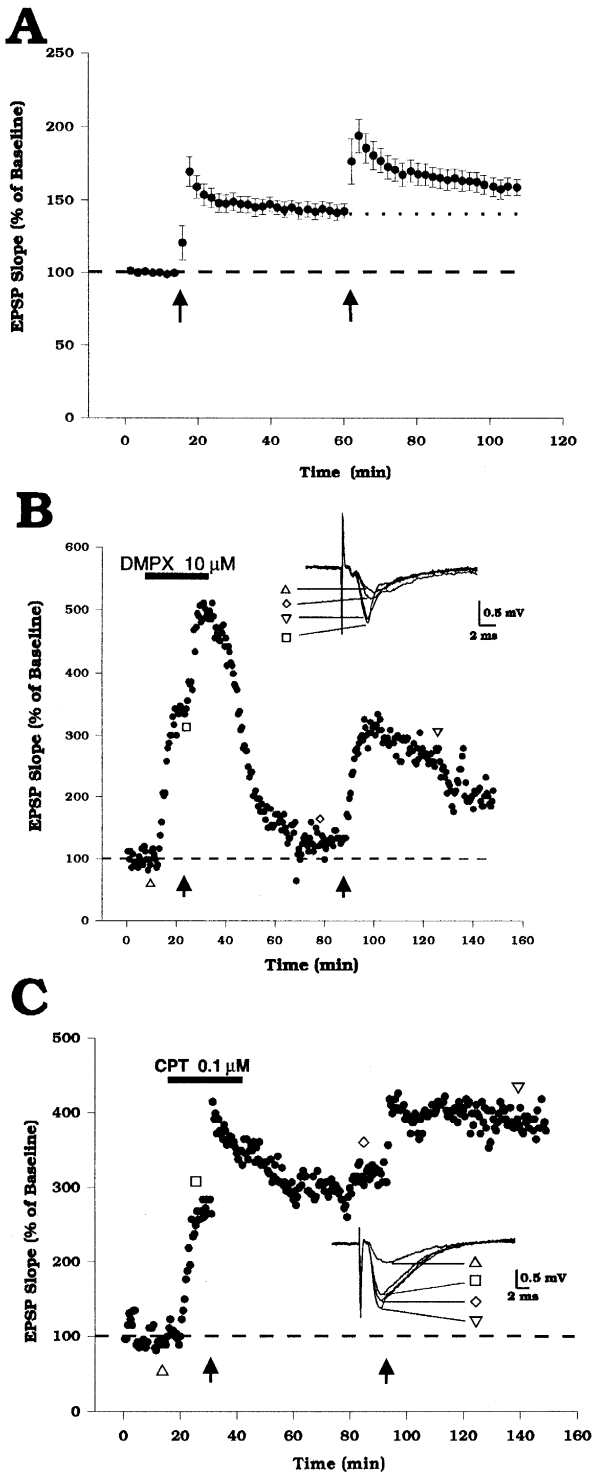

Fig. 2. DMPX reduces LTP induction. A: under control conditions in aCSF, two tetani were applied at 45 min intervals. Plot shows the average EPSP slope ($n = 8$) recorded relative to the original baseline indicating that significant potentiation could still be elicited with this tetanic stimulation after a second tetanus. After the first tetanus, EPSP was potentiated by 41.9 $\pm$ 2.2% (mean $\pm$ S.E.M.); after the second tetanus, by 58.8 $\pm$ 2.7% at the end of 45 min compared to the original baseline level. B: a stable baseline EPSP slope was recorded in control aCSF during orthodromic stimulation. Addition of DMPX increased the EPSP slope as seen in Fig. 1A. Potentiation in response to a tetanus (100 Hz, 1 s; indicated by vertical arrows) in the presence of DMPX was short-lived and, following rapid washout, the EPSP returned to near baseline levels. Application of a second tetanus yielded LTP. Inset: field potential recordings. C: exposure to CPT caused the EPSP to rise above baseline levels. A 1 s, 100 Hz tetanus (arrows) caused a potentiation which remained elevated for 55 min after washout of CPT. Delivery of a second tetanus caused an additional potentiation. Inset: field potential recordings.

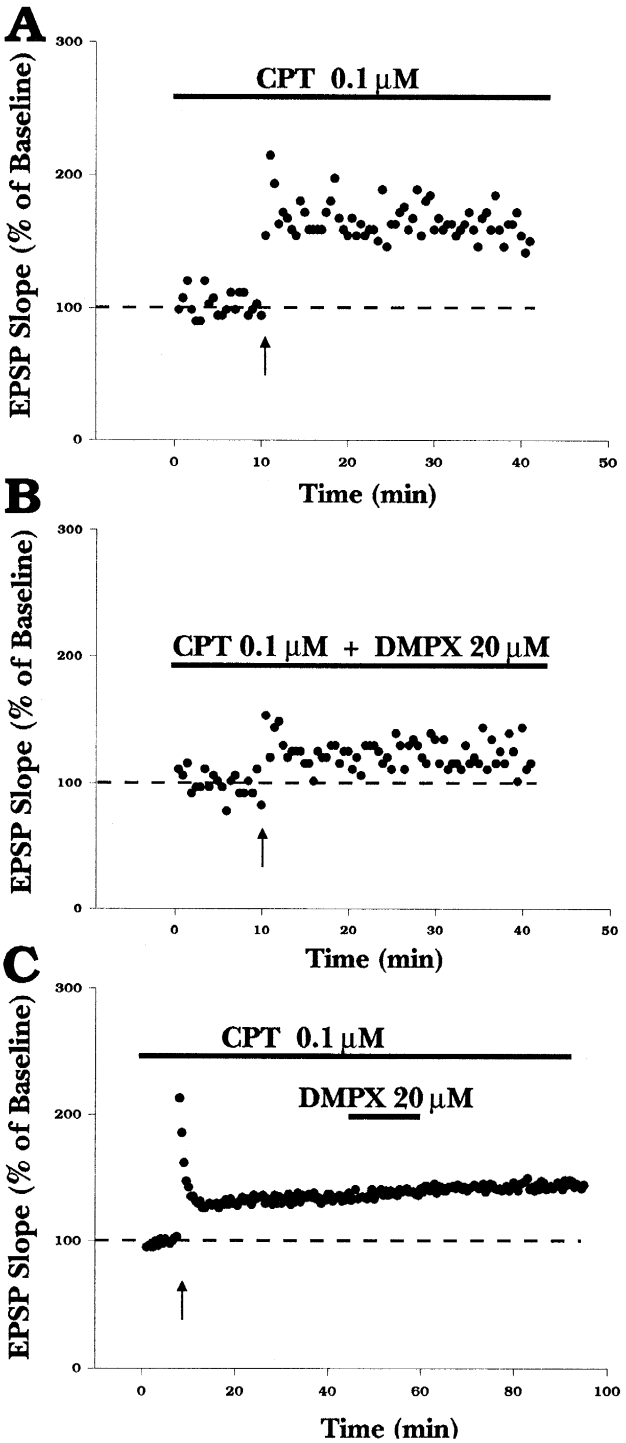

Fig. 3. A_2 receptor blockade inhibits LTP induction but not expression. A: control protocol in which tetanus-induced LTP occurs in the presence of the A_1 receptor blocker, CPT. B: in the presence of both CPT and DMPX, tetanus-induced LTP induction is significantly reduced (see Section 3) over that seen with CPT alone. C: DMPX does not diminish LTP expression. Arrows indicate a 1 s, 100 Hz tetanus throughout. Application of a tetanus in CPT increased the average ($n = 6$) EPSP level to 53% over the baseline level recorded under control conditions. Application of DMPX to this slice resulted in no observable decrease in the potentiated EPSP level.

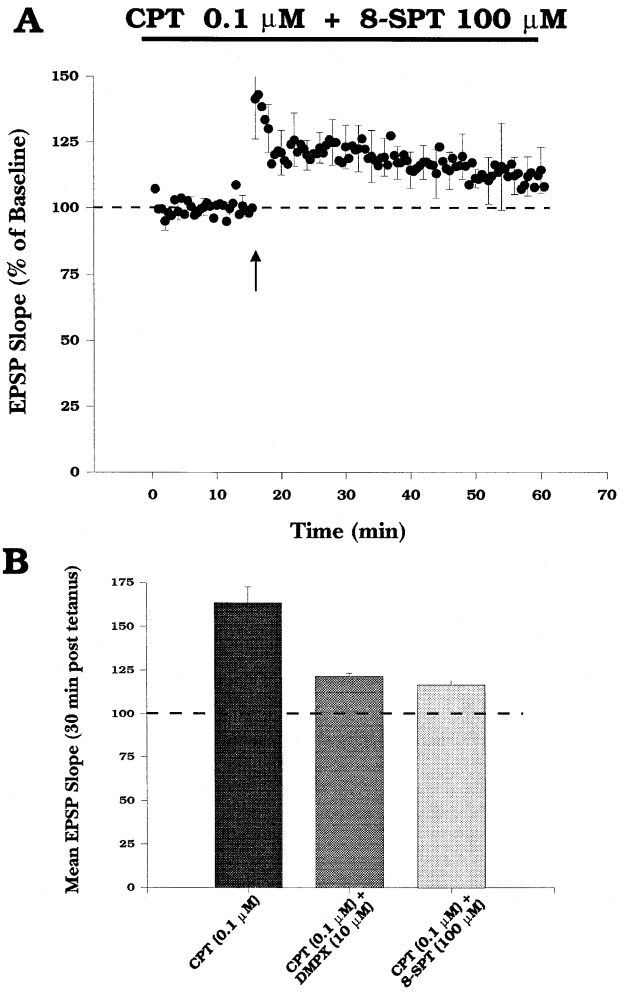

Fig. 4. Blockade of A_2 receptors with an impermeant antagonist also reduces tetanus-induced LTP. A: in the presence of both A_1 and A_2 blockade with CPT and the polar antagonist 8-SPT, a brief tetanus produces a reduced level of LTP compared to that observed with CPT alone (see Fig. 3A). B: comparison of the level of LTP 30 min post tetanus in CPT alone or with either DMPX or 8-SPT. No statistically significant difference was observed ($P > 0.7$) between the level of LTP induced using the two different A_2 antagonists.

3.2. DMPX inhibits induction of LTP by blocking a non-A_1 adenosine receptor

Because A_2 receptors play a potentiating role in normal synaptic transmission, we examined the effects of these receptors on tetanus-induced LTP. In preliminary experiments we measured the mean potentiation 45 min following each of two tetani during perfusion of control buffer. The average level of potentiation ($n = 8$) following each of two tetani (Fig. 2A) were 41.9% and 58.8% above the baseline, respectively, indicating that significant potentiation could be reliably induced by both a first and second tetanus. In separate experiments we pre-exposed slices to either DMPX or CPT for 10 min prior to tetanic stimula-

tion, followed by a washout of the drug for at least 40 min. During bath perfusion of DMPX, application of a tetanus resulted in a brief post-tetanic potentiation which decreased to near baseline levels during washout (Fig. 2B). In contrast, a second tetanus in the absence of DMPX resulted in LTP as measured by sustained potentiation of the EPSP 45 min post tetanus.

Because the removal of DMPX occurred within 5 min after tetanus, it could be argued that the effects on LTP with this protocol might be confused by the return of A_1 inhibition soon after washout. Therefore, a second series of experiments was performed in which exposure to DMPX was maintained at least 30 min following the tetanus. Although the EPSP remained elevated during DMPX exposure relative to the control baseline due to its antagonism of A_1 receptors, following washout the mean EPSP returned to within 15% of the original control baseline within 18 min consistent with the shorter exposure to the A_2 antagonist further indicating that the A_2 blockade was responsible for the lack of LTP induction (result not shown). To exclude the possibility that the effects of this drug on LTP were a result of A_1 receptor blockade, we examined the effect of the highly selective A_1 receptor antagonist, CPT. Consistent with previous reports, A_1 receptor blockade further increased the level of LTP even 45 min after washout (Fig. 2C). In control experiments, the EPSP elicited during low frequency test pulses returned to within 9% of baseline levels 45 min following washout of 0.1 μM CPT suggesting that the elevated effects were not substantially due to significant residual binding of CPT. Application of a second tetanus following washout induced a further increase in the level of LTP. The larger tetanus-induced potentiation observed during A_1 blockade compared to control is consistent with previously published reports [6] that demonstrated a disinhibition of synaptic transmission and LTP following A_1 receptor blockade. Together, these results suggest that DMPX blocks LTP by interacting with an A_2 receptor.

3.3. DMPX suppresses LTP in the presence of A_1 receptor blockade

In order to isolate the effects of DMPX on LTP mediated by A_2 receptors, we compared the effects of perfusing either CPT alone or DMPX and CPT on LTP. After establishing a baseline of stable responses for at least 10 min, a tetanus was applied and the resulting potentiation monitored for 30 min. The level of LTP was determined as the mean potentiation of synaptic responses during the last 10 min of each experiment. In the presence of CPT alone, the level of LTP attained (Fig. 3A) was $163.5 \pm 9\%$ ($n = 5$). However, in separate experiments, when DMPX and CPT were perfused together, the level of LTP attained (Fig. 3B) was reduced to $121.3 \pm 2\%$ ($n = 6$) when compared to that during exposure to CPT alone.

Because the initiation and maintenance of LTP are believed to occur via separate mechanisms (for review: [2]), the question arises as to whether the suppression of LTP by DMPX was a result of a disruption of the induction or maintenance phases. To investigate whether A_2 receptors play a role in the maintenance of LTP, DMPX (10 μM) was applied to a slice following tetanus-induced

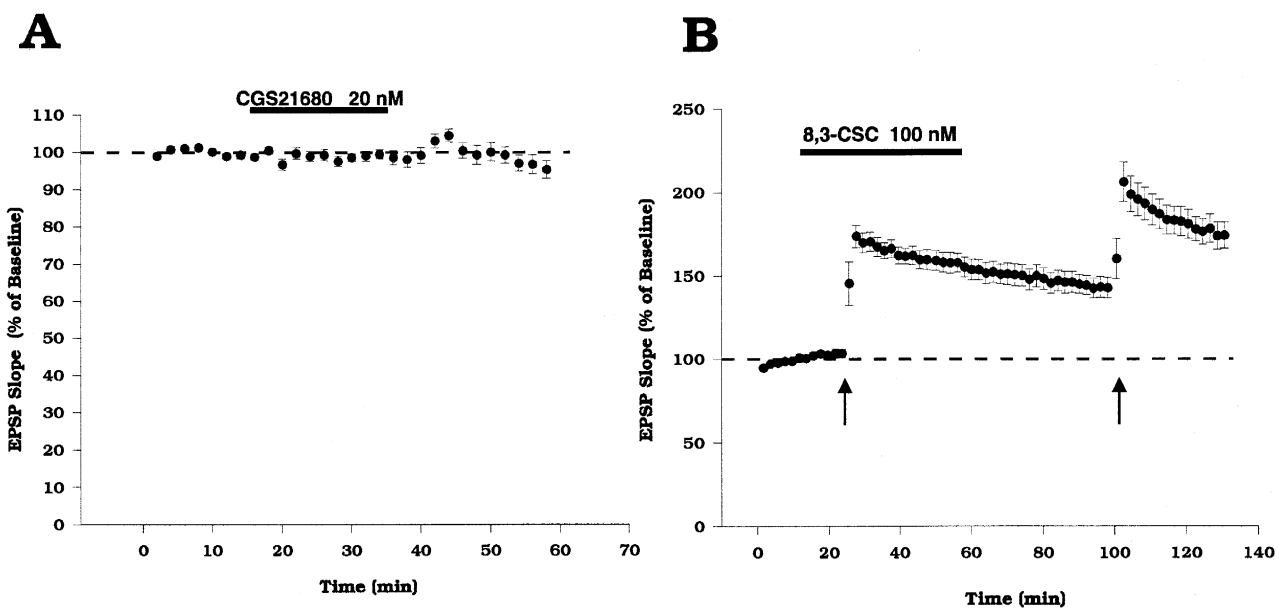

Fig. 5. A_{2a} receptors are not involved in modulation of synaptic transmission. A: the highly selective A_{2a} agonist, CGS 21680, was applied to a slice during low-frequency test pulses (0.033 Hz). No effect of the agonist was observed on the field EPSP in $n = 6$ experiments. B: the time course of the average response ($n = 4$) of the field EPSP to 1 s, 100 Hz tetani (arrows) is plotted during or after exposure to the selective A_{2a} receptor inhibitor, 8,3-CSC. A tetanus applied in the presence of the drug did not inhibit LTP up to 75 min after tetanic stimulation. Following washout, a second tetanus induced additional potentiation.

LTP. Cells were exposed to CPT (0.1 μM) throughout the experiment to eliminate the effects of DMPX on A_1 receptors (Fig. 3C). The potentiated EPSP observed in response to DMPX applied 25 min post tetanus was not significantly altered with an average decrease of $7.2\% \pm 2.5\%$ ($n = 9$) consistent with that seen under non-tetanized control conditions (Fig. 1B) indicating that the effects of A_2 blockade on LTP occurred during the critical induction period and not during the maintenance phase.

The possibility remains that the effect of DMPX on the reduction of tetanus-induced LTP may have been occurring by an intracellular action of the drug such as by an antagonism of a phosphodiesterase rather than through blockade of an external adenosine receptor. Therefore, we tested the effect of A_2 receptor blockade on LTP using a highly polar A_2 receptor antagonist with no known intracellular activity. Fig. 4A shows the time course of the response of the EPSP to a tetanus in the presence of the CPT and the A_2 antagonist 8-SPT (100 μM). Using the same protocol as in Fig. 3B, a tetanus in 8-SPT resulted in LTP equal to $116.4 \pm 2.6\%$ ($n = 5$). A comparison of the two methods of A_2 blockade with that of control is shown in Fig. 4B indicating that the two A_2 receptor antagonists produced nearly identical results ($P > 0.7$; *t*-test) suggesting that the effects of DMPX were through its blockade of the A_2 receptor.

3.4. A_{2a} receptors are not involved in the DMPX effects

Adenosine A_2 receptors have been divided into two subclasses, designated as the A_{2a} and A_{2b} receptors [4,23]. Although no specific A_{2b} receptor agonists and antagonists are currently available, highly specific agents for the A_{2a} receptor do exist and were utilized to determine the contribution of this receptor in the influence of DMPX on synaptic transmission and LTP.

Bath application of the selective A_{2a} agonist, CGS21680 (5–30 nM), was incapable of inducing enhancement of the field EPSP in response to low frequency test pulses (Fig. 5A), an effect similar to that seen by Sebastiao and Ribeiro [21]. Low concentrations of CGS21680 were used because higher concentrations have been found to produce antagonist effects [21]. In contrast, tetanus-induced LTP induction may require a prior activation of some mechanism before A_{2a} receptors can contribute to an increase in synaptic efficacy. Therefore, the effect of specific A_{2a} receptor blockade on LTP induction was examined using a similar experimental protocol to that used when examining the effects of DMPX. Fig. 5B shows that the selective A_{2a} receptor antagonist, 8,3-CSC (100 nM), did not block LTP induction. As in control conditions, both the first and second tetani were capable of inducing LTP although any longer-term effect of A_{2a} blockade beyond 75 min was not examined in this study. These results suggest that the effects of DMPX on LTP induction were independent of the A_{2a} receptor.

4. Discussion

Our experiments suggest that A_2 receptor activation both enhances normal synaptic transmission and contributes to tetanus-induced LTP. Adenosine concentration in the extracellular fluid of the brain is proportional to the level of neuronal firing. This elevation is due, at least in part, to the co-release of ATP with other neurotransmitters such as acetylcholine and catecholamines [1,5,19,25]; ATP is then rapidly broken down to adenosine by ectonucleotidases [18,26]. Whether activity-dependent increases in adenosine are due to this mechanism or to a direct release of adenosine from neurons, synapses experiencing increased activity probably experience focal 'hot spots' of adenosine concentrations above basal levels in the clefts.

Our finding that blockade of an A_2 receptor with DMPX in the absence of A_1 receptor activity depresses the field EPSP suggests that A_2 receptors may contribute to synaptic transmission under normal physiological conditions. Such a contribution could be either through an increase in transmitter release or an increase in postsynaptic responsiveness. In support of a presynaptic mechanism is the finding that exogenously applied adenosine increases transmitter release in the hippocampus [16] and also differentially modulates Ca^{2+} current in CA3 pyramidal neurons [14] whose axons form the Schaffer collateral pathway. In contrast, a postsynaptic mechanism which increases the excitability of CA1 neurons could potentially account for the observed effects. For example, it is known that A_2 receptors increase the levels of intracellular cAMP [9] and also that PKA, the cAMP-dependent protein kinase, increases the sensitivity of AMPA receptors [11] which mediate the fast synaptic responses of CA1 neurons.

Induction and expression of LTP are believed to proceed along different pathways [2]. Because DMPX significantly suppresses LTP when applied during the LTP induction protocol but has no effect on the expression of LTP suggests that DMPX interacts with the mechanisms that contribute to the induction of LTP. However, the possibility that expression of LTP can be modulated only during or immediately following tetanic stimulation cannot be excluded. We have shown that the effect of DMPX on LTP induction is independent of any influence on adenosine A_1 receptors. The fact that DMPX has been characterized as an A_2 receptor blocker and that selective activation and blockade of the A_{2a} receptor did not affect LTP induction suggests that the A_{2b} receptor was responsible for the effects observed. This hypothesis is consistent with the pharmacological evidence for A_{2b} involvement in Ca current potentiation [14] although the possibilities that DMPX may have cross-affinity with another receptor subtype or that the effect of A_2-induced Ca current potentiation is independent of the effects on neurotransmission cannot be excluded.

Although our experiments do not explore the site of action of A_2 receptors, the suggestion that these receptors

interfere with induction mechanisms is consistent with a postsynaptic site of action [13,24]. Recently it has been reported that elevated cAMP levels are critical for the induction of LTP in the CA1 region [3]. Because A_2 receptors are known to elevate cAMP levels [9], it is possible that these receptors exert their effects on LTP by modulating the levels of cAMP. The fact that different patterns of afferent stimulation induce different forms of LTP in the hippocampus [12] and that the effect on LTP induction by A_2 blockade is dependent on the strength of tetanic stimulation [10] suggests that adenosine may play only a partial role in the overall phenomenon. Further study of the relative distribution of the different adenosine receptor subtypes and the different sources of extracellular adenosine will help to clarify these issues.

Acknowledgements

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