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Potentiation of excitatory amino acid-evoked adenosine release from rat cortex by inhibitors of adenosine kinase and adenosine deaminase and by acadesine

Thomas D. White *

Department of Pharmacology, Dalhousie University, Halifax, Nova Scotia B3H 4H7, Canada

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Abstract

Endogenous extracellular adenosine provides some protection against excitotoxicity in the central nervous system, but it appears to be incomplete. Potentiating the formation of extracellular adenosine that occurs when excitatory amino acid receptors are activated might provide additional protection. We studied the effects of AICAR (AICA riboside, acadesine) and of inhibitors of adenosine metabolism on the release of adenosine from rat cortical slices. AICAR had no effects on basal *N*-methyl-D-aspartate (NMDA)- or (*RS*)-α-amino-3-hydroxy-5-methyl-4-isoxasole propionic acid (AMPA)-evoked adenosine release, but it increased kainate-evoked adenosine release 1.4-fold. This selective action of AICAR may make it useful for treating kainate receptor-mediated excitotoxicity. Inhibition of adenosine kinase with either 20 μM 5′-amino-5′-deoxyadenosine or 5′-iodotubercidin had a much greater effect on excitatory amino acid-evoked adenosine release than on basal adenosine release. Inhibition of adenosine kinase increased excitatory amino acid-evoked adenosine release 3–7-fold whereas inhibition of adenosine deaminase only increased evoked adenosine release 2–2.5-fold. Finally, 0.2 μM 5′-iodotubercidin and 200 μM 2′-deoxycoformycin caused similar increases in the basal rates of extracellular adenosine formation, but 5′-iodotubercidin produced over twice as much potentiation of the rate of NMDA-evoked adenosine formation than did 2′-deoxycoformycin. These findings suggest that adenosine kinase inhibitors may produce an event-specific potentiation of evoked adenosine formation, i.e. more effect on evoked formation than on basal formation. If so, adenosine kinase inhibitors may prove useful for preventing/treating diseases associated with excessive excitation in the brain, such as seizures, excitotoxicity and neurodegeneration.

Keywords: NMDA (*N*-methyl-D-aspartate); AMPA (α-amino-3-hydroxy-5-methyl-4-isoxasole propionic acid); Kainate; Adenosine kinase; Adenosine deaminase; AICAR (AICA riboside, acadesine)

1. Introduction

In conditions, such as seizure disorders, ischemia, hypoxia and certain neurodegenerative disorders, some of the neuronal damage appears to be due to excessive excitatory amino acid-mediated neurotransmission (Lipton and Rosenberg, 1994). Extracellular adenosine, acting at specific adenosine receptors, exerts important inhibitory effects in the brain (reviewed in Abbracchio and Cattabeni, 1993; Belardinelli and Pelleg, 1994). By depressing both the release and the postsynaptic actions of glutamate, extracellular adenosine modulates excitatory amino acid-mediated neurotransmission (Dolphin and Archer, 1983; Drejer et al., 1987). Activation of excitatory amino acid receptors releases adenosine from rat cortex in vitro (Hoehn and White, 1990a, b) and there are spare NMDA receptors for adenosine release (Hoehn et al., 1990; Craig and White, 1991, 1992, 1993a). The presence of spare receptors explains why NMDA is 33 × more potent at releasing adenosine than at releasing noradrenaline. The observation that maximal adenosine release occurs when only a few of the available NMDA receptors are activated, prompted us to suggest that the primary function of this released adenosine was to provide an inhibitory threshold against normal NMDA receptor-mediated neurotransmission in the brain, rather than to protect against excessive NMDA receptor activation. Indeed the results of two recent electrophysiological studies indicate that adenosine, released as a result

* Tel.: (1) (902) 494-3462; fax: (1) (902) 494-1388; e-mail: tom.white@dal.ca

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of low level activation of NMDA receptors during glutamatergic transmission, acts presynaptically to decrease the release of glutamate and depress excitation in the CA1 region of the hippocampus (Mitchell et al., 1993; Manzoni et al., 1994). Activation of either AMPA or kainate receptors releases adenosine per se whereas activation of NMDA receptors releases a nucleotide, possibly ATP, which is then converted to adenosine extracellularly by ectonucleotidases (Craig and White, 1993a, b; Craig et al., 1994). Other researchers have shown that a variety of stimuli release adenosine into the extracellular space in the brain in vivo (Jonzon and Fredholm, 1985; Chen et al., 1992; Sciotti et al., 1992, 1993; Pazzagli et al., 1994).

Studies with adenosine A_1 receptor antagonists indicate that endogenous extracellular adenosine provides some protection against excessive excitatory amino acid-mediated stimulation and seizures (Dragunow and Robertson, 1987; Dragunow, 1991; Von Lubitz et al., 1993; Young and Dragunow, 1994). However, this protection by endogenous adenosine appears to be incomplete, in so far as supplementation with adenosine receptor agonists provides additional protection against seizures (Von Lubitz et al., 1993). Unfortunately, the development of clinically useful adenosine A_1 receptor agonists has been hampered by numerous unacceptable side-effects, apparently related to the actions of these agents at adenosine receptors located in many tissues and organs throughout the body. Another approach is to potentiate the levels of endogenous extracellular adenosine in an event-specific manner, that is to potentiate evoked formation of extracellular adenosine more than basal formation (see Fig. 1 for the metabolic pathways for adenosine). The primary advantage of this approach is that the potentiation of extracellular adenosine levels may be restricted primarily to those sites where endogenous extracellular adenosine formation is greatest, for instance in hypoxic/ischemic tissues or where excessive stimulation occurs. Uptake of extracellular adenosine can be inhibited by dipyridamole, nitrobenzylthioinosine and dilazep, which block the adenosine transporter. Conversion of adenosine to 5'AMP can be prevented by 5'-amino-5'-deoxyadenosine (NH_2dADO) and by 5'-iodotubercidin, which inhibit adenosine kinase. Conversion of adenosine to inosine can be prevented by EHNA (erythro-9-(2-hydroxy-3-nonyl) adenine) and by 2'-deoxycoformycin, which inhibit adenosine deaminase. The natural product AICAR (AICA riboside, acadesine, 5'-amino-1-(5-*O*-phosphono-β-D-ribofuranosyl)-1 *H*-imidazole-4-carboxamide) is a very weak inhibitor of adenosine kinase and

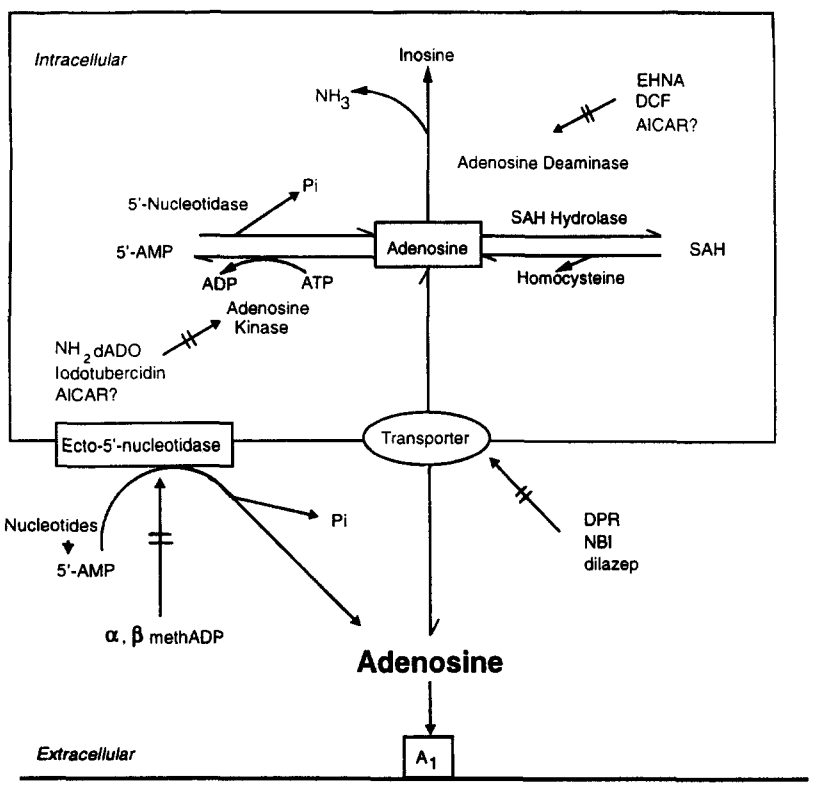

Fig. 1. Metabolic routes for adenosine metabolism and the sites of action of various inhibitors in the CNS (derived from Zhang et al., 1993). In the brain, metabolism by adenosine kinase appears to be a major route of removal of adenosine. Inhibitors of adenosine kinase include 5'-amino-5'-deoxy adenosine (NH_2dADO), 5'-iodotubercidin and possibly AICAR (weak). Inhibitors of adenosine deaminase include EHNA, 2'-deoxycoformycin (DCF) and possibly AICAR (weak). Inhibitors of adenosine transport include dipyridamole (DPR), nitrobenzylthioinosine (NBI) and dilazep. Ecto-5'-nucleotidase is inhibited by α,β-methylene ADP (α,βmethADP).

adenosine deaminase. It has been used to increase extracellular adenosine selectively (in an event-specific manner) in ischemic regions and provide protection against ischemic damage in animal hearts (Gruber et al., 1989; Mullane, 1993; Mullane and Young, 1993).

Several groups have studied the effects of adenosine kinase and adenosine deminase inhibitors on the release of adenosine in the central nervous system (CNS) (Ballarin et al., 1991; Sciotti and Van Wylen, 1993; Pak et al., 1994; Lloyd and Fredholm, 1995). In the present study, we attempted to potentiate the formation of extracellular adenosine that occurs when excitatory amino acid receptors are activated. Specifically, we determined the effects that inhibitors of adenosine kinase and adenosine deaminase, and AICAR have on the amounts of extracellular adenosine formed when NMDA, AMPA or kainate receptors are activated in rat cortical slices. The results indicate that the pharmacological potentiation of the formation of endogenous extracellular adenosine in response to excitatory amino acid receptor stimulation may have important therapeutic potential for treating seizures, excitotoxicity and neurodegenerative disorders. Portions of this research have been presented previously in preliminary forms (White, 1994a, b).

2. Materials and methods

2.1. Preparation of cortical slices

Male Sprague-Dawley rats (250–350 g; Charles River Canada, St. Constant, Quebec, Canada) were killed by decapitation and their brains rapidly excised into ice-cold Krebs-Henseleit bicarbonate buffer containing (mM) 111 NaCl, 26.2 $NaHCO_3$, 1.2 NaH_2PO_4, 4.7 KCl, 1.8 $CaCl_2$, 1.2 $MgCl_2$, 11 glucose, gassed with 95% O_2-5% CO_2 to maintain a pH of 7.4. The outer 1–1.5-mm layer of the parietal cortex was removed from both hemispheres with a recessed tissue slicer and 0.4-mm coronal slices were prepared with a McIlwain tissue chopper. Adjacent slices were placed alternately into two tissue baths so that each bath contained six slices (three from the left and three from the right hemispheres).

2.2. Superfusion of slices

The slices were placed onto nylon mesh screens supported by cylindrical platforms in two tissue baths adjusted to internal volumes of 0.5 ml. The two baths were run in parallel and were assigned in alternate experiments as either 'test' or 'control' treatments. Slices were superfused from top to bottom with oxygenated Krebs-Henseleit buffer at 36°C and a flow rate of 0.75 ml/min. Slices were equilibrated for 65 min prior to collection of 10 serial 2.5-min fractions. Three initial fractions were collected to determine basal adenosine release, then the superfusing buffer was switched for 10 min to buffer containing releasing agent (100 μM, the EC_{90} for NMDA, kainate or AMPA) after which the superfusing buffer was switched back to Krebs-Henseleit buffer for the final three fractions. The various inhibitors of adenosine kinase, adenosine deaminase, or AICAR were introduced into the superfusing buffer 10 min prior to collection of the first fraction and continued until the end of the experiment. Following collection of all fractions, slices were removed from the tissue baths and weighed.

2.3. Determination of adenosine release

Samples of superfusate were deproteinated with $Ba(OH)_2$ and $ZnSO_4$ and then reacted with chloroacetaldehyde to form 1-N6-ethenoadenosine, which was assayed by HPLC with fluorescence detection as described previously (Hoehn and White, 1990a). Adenosine standards were prepared in appropriate drug containing Krebs-Henseleit solutions and processed identically to superfusate samples. The sample adenosine content was quantitated by peak height and compared with the standards. Rate of adenosine release for each fraction is expressed as pmol/g cortex/min. Evoked adenosine release (above basal) was obtained by subtracting the rate of release for the sample immmediately preceding exposure to releasing agent from that for all other samples. Total evoked adenosine release was determined as the cumulative amount of evoked adenosine released following exposure to the releasing agent and is expressed as nmol/g of cortex.

2.4. Drugs

Adenosine, NMDA (*N*-methyl-D-aspartate), kainic acid, AICAR (5′-amino-1-(5-*O*-phosphono-β-D-ribofuranosyl)-1*H*-imidazole-4-carboxamide) and 5′-amino-5′-deoxyadenosine (NH_2dADO) were purchased from Sigma (St. Louis, MO), 5′-iodotubercidin and EHNA (erythro-9-(2-hydoxy-3-nonyl)adenine hydrochloride) from Research Biochemicals International (Natick, MA), AMPA ((*RS*)-α-amino-3-hydroxy-5-methyl-4-isoxazole propionic acid) from Tocris Cookson (Bristol, UK) and choloroacetaldehyde from Aldrich (Milwaukee, WI). 2′-Deoxycoformycin (DCF, Pentostatin) was a gift from Parke-Davis Pharmaceutical Research Division (Ann Arbor, MI). AICAR and 5′-iodotubercidin were dissolved in dimethyl sulfoxide and diluted 500-fold in Krebs-Henseleit medium. All other drugs dissolved freely in Krebs-Henseleit medium. Controls were superfused identically with corresponding solvent-containing media.

2.5. Statistical analysis

The effects of the drugs on basal rates of adenosine release and on the maximal rates of evoked adenosine release from two concurrently run control and test tissue

baths were analysed for differences by Student's t test for paired data ($P < 0.05$). The same statistical test was also used to determine the effects that the drugs had on the total amounts of adenosine released during exposure to NMDA, AMPA or kainate. Unpaired t tests were performed when comparing data from separate experiments.

3. Results

3.1. Effects of inhibitors of adenosine kinase on extracellular adenosine levels following the activation of NMDA, AMPA and kainate receptors in cortical slices

Inhibition of adenosine kinase with 20 μM 5′-amino-5′-deoxyadenosine (NH_2dADO) increased the basal rate of adenosine release 1.7-fold (from 111 ± 8 to 185 ± 13 pmol/g/min, $n = 13$, $P < 0.05$) but increased the maximal rates of evoked release of adenosine 2.5-fold (from 337 ± 20 to 851 ± 189 pmol/g/min, $n = 5$, $P < 0.05$) for NMDA, 2.3-fold (from 262 ± 34 to 605 ± 53 pmol/g/min, $n = 4$, $P < 0.05$) for AMPA and 2.7-fold (from 191.3 ± 10.9 to 511.3 ± 54.9 pmol/g/min, $n = 4$, $P < 0.05$) for kainate (Fig. 2). The total amounts of adenosine released by NMDA, AMPA and kainate were increased 3.7-, 3.1- and 4.1-fold, respectively, by 5′-amino-5′-deoxyadenosine.

Inhibition of adenosine kinase with 20 μM 5′-iodotubercidin (Fig. 3) increased the basal rate of adenosine release 3.6-fold (from 106 ± 10 to 383 ± 15 pmol/g/min, $n = 10$, $P < 0.05$) but increased the maximal rates of evoked adenosine release 4.5-fold (from 292 ± 34 to 1303 ± 97 pmol/g/min, $n = 4$, $P < 0.05$) for NMDA, 4.1-fold (from 254 ± 3 to 1037 ± 74 pmol/g/min, $n = 3$, $P < 0.05$) for AMPA and 4.1-fold (from 215 ± 47 to 887 ± 105 pmol/g/min, $n = 3$, $P < 0.05$) for kainate. The total amounts of adenosine released by NMDA, AMPA and kainate were increased 5.8-, 5.2- and 7.4-fold, respectively, by 5′-iodotubercidin.

These observations indicate that inhibition of adenosine kinase has a greater effect on excitatory amino acid-evoked adenosine release than on basal release, suggesting that the potentiation of adenosine formation exhibited event-specificity.

3.2. Effects of inhibitors of adenosine deaminase on extracellular adenosine levels following the activation of NMDA, AMPA and kainate receptors in cortical slices

Inhibition of adenosine deaminase with 20 μM EHNA (Fig. 4) slightly increased the basal rate of adenosine release 1.3-fold (from 127 ± 9 to 161 ± 10 pmol/g/min, $n = 16$, $P < 0.05$) and appeared to slightly increase the maximal rate of EAA-evoked adenosine release, although only the effect on the rate of kainate-evoked adenosine release was statistically significant (1.5-fold, increased from 244 ± 27 to 363 ± 34 pmol/g/min, $n = 3$, $P < 0.05$). The total amounts of adenosine released by NMDA, AMPA and kainate appeared to be increased slightly by EHNA but only the effect on kainate-evoked release was significant (2.1-fold increase).

Inhibition of adenosine deaminase with a high dose (200 μM) of 2′-deoxycoformycin (Fig. 5) increased the basal rate of adenosine release 1.6-fold (from 146 ± 8 to 232 ± 18 pmol/g/min, $n = 10$, $P < 0.05$) and increased the maximal rates of evoked adenosine release 1.8-fold (from 375 ± 33 to 677 ± 77 pmol/g/min, $n = 4$, $P < 0.05$) for NMDA, 1.7-fold (from 282 ± 19 to 465 ± 11 pmol/g/min, $n = 3$, $P < 0.05$) for AMPA and 1.6-fold (from 264 ± 25 to 426 ± 56 pmol/g/min, $n = 3$, $P < 0.05$) for kainate. The total amounts of adenosine released by NMDA, AMPA and kainate were increased 2.5-, 1.9- and 1.9-fold, respectively, by this large dose of 2′-deoxycoformycin.

These observations indicate that inhibition of adenosine deaminase potentiates both basal and excitatory amino acid-evoked formation of extracellular adenosine.

3.3. Effects of AICAR on extracellular adenosine levels following the activation of NMDA, AMPA and kainate receptors in cortical slices

AICAR (20 μM) had no effect on the basal rate of adenosine release, nor did it affect the release of adenosine evoked by either NMDA or AMPA (Fig. 6). However, AICAR did increase the maximal rate of adenosine release evoked by kainate 1.4-fold (from 184 ± 29 to 254 ± 39, $n = 4$, $P < 0.05$) and the total amount of adenosine released by kainate was increased 2.1-fold. These findings suggest that AICAR has moderate potentiating effects that are selective for kainate-evoked adenosine release.

3.4. Comparison of the effects of adenosine kinase inhibition with adenosine deaminase inhibition on basal and NMDA-evoked rates of formation of extracellular adenosine

In order to compare the effects of adenosine kinase inhibition with the effects of adenosine deaminase inhibition on extracellular adenosine formation, we first determined doses of 5′-iodotubercidin and 2′-deoxycoformycin that produced similar potentiations of the basal rates of adenosine formation. We found that 0.2 μM 5′-iodotubercidin increased the basal rate of extracellular adenosine to a similar extent as 200 μM 2′-deoxycoformycin (increased by 78.5 ± 8.9 pmol/g/min for 5′-iodotubercidin vs. 86.1 ± 44.4 for 2′-deoxycoformycin, no difference, unpaired t test). However, at these doses 5′-iodotubercidin

increased the maximal rate of NMDA-evoked adenosine formation by 784 ± 67 pmol/g/min, whereas 2′-deoxycoformycin only increased the maximal rate of NMDA-evoked adenosine formation by 303 ± 59 pmol/g/min, significantly less than the effect produced by 5′-iodotubercidin ($P < 0.05$, unpaired t test). This suggests that the event-specificity of adenosine kinase inhibition may be greater than adenosine deaminase inhibition.

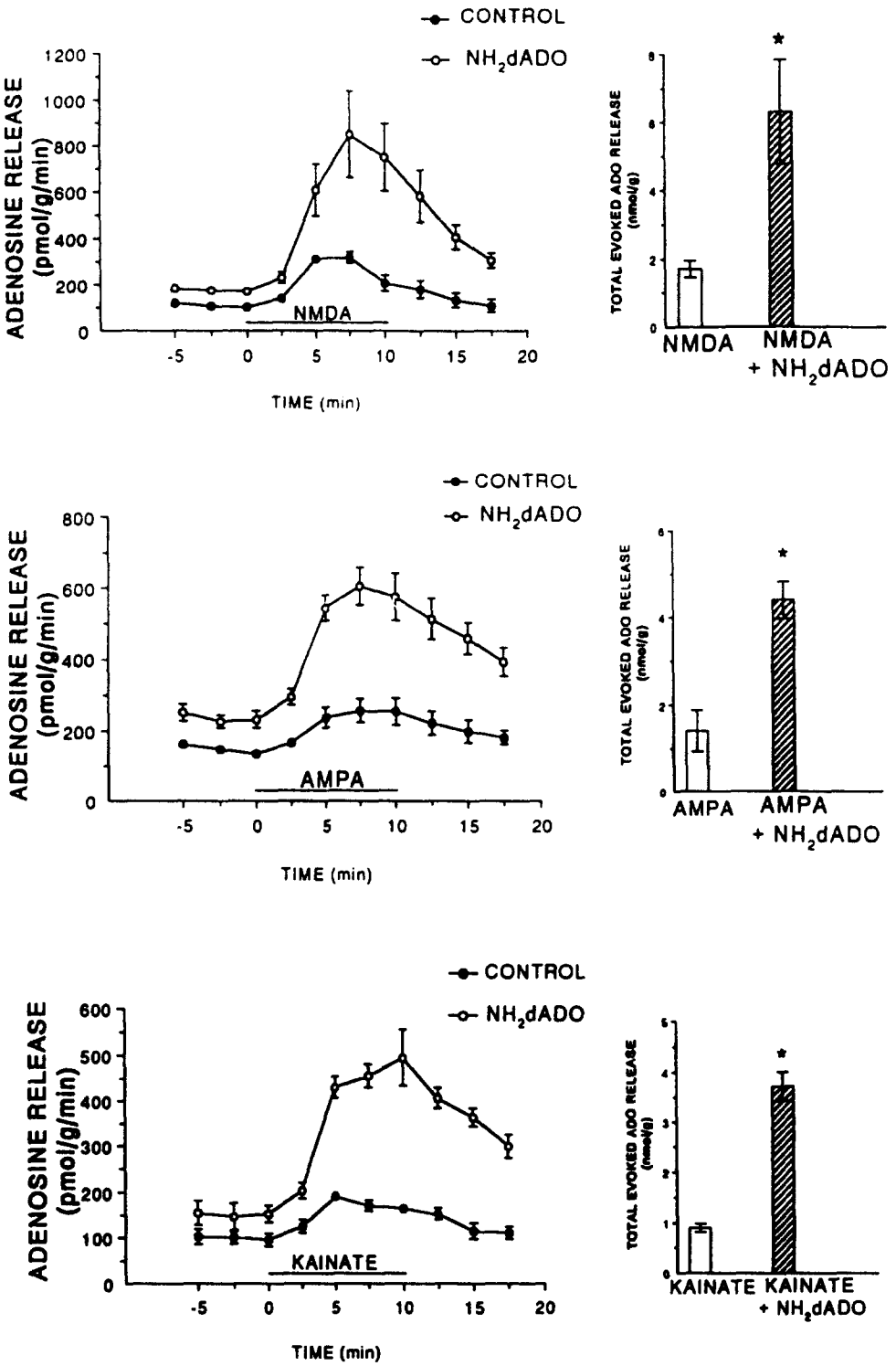

Fig. 2. Effect of inhibition of adenosine kinase with a supramaximal concentration of 5′-amino-5′-deoxyadenosine (NH_2dADO, 20 μM) on the formation of extracellular adenosine in rat cortical slices. Upper panel shows release of adenosine evoked by 100 μM NMDA ($n = 5$), middle panel shows release of adenosine evoked by 100 μM AMPA ($n = 4$), lower panel shows release of adenosine evoked by 100 μM kainate ($n = 4$). * Significantly different from control ($P < 0.05$, paired t test).

4. Discussion

The results of the present study indicate that inhibition of adenosine kinase produces an event-specific potentiation of excitatory amino acid-evoked formation of extracellular adenosine, in so far as the effects on excitatory amino acid-evoked release are much greater than the effects on basal adenosine release. Thus, inhibition of adenosine ki-

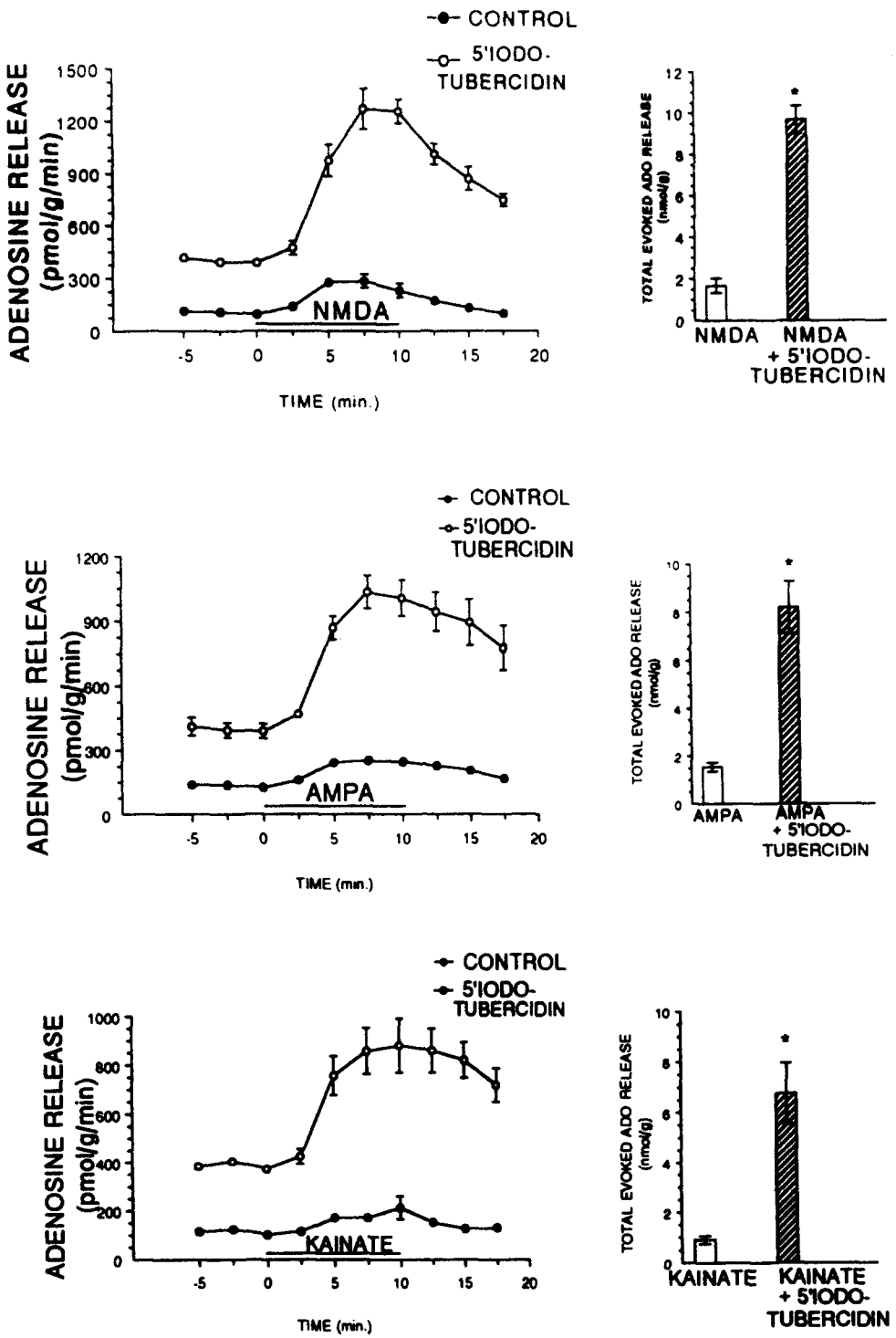

Fig. 3. Effect of inhibition of adenosine kinase with a supramaximal concentration of 5'-iodotubercidin (20 μM) on the formation of extracellular adenosine in rat cortical slices. Upper panel shows release of adenosine evoked by 100 μM NMDA ($n = 4$), middle panel shows release of adenosine evoked by 100 μM AMPA ($n = 3$), lower panel shows release of adenosine evoked by 100 μM kainate ($n = 3$). * Significantly different from control ($P < 0.05$, paired t test).

nase with 20 μM 5′-amino-5′-deoxyadenosine increased the basal rate of adenosine release 1.7-fold but increased the maximal rates of evoked adenosine release 2.5-fold for NMDA, 2.3-fold for AMPA and 2.7-fold for kainate. Similarly, inhibition of adenosine kinase with 20 μM 5′-iodotubercidin increased the basal rate of adenosine release 3.6-fold but increased the maximal rates of evoked adenosine release 4.5-fold for NMDA, 4.1-fold for AMPA and 4.1-fold for kainate.

Inhibition of adenosine deaminase with 20 μM EHNA

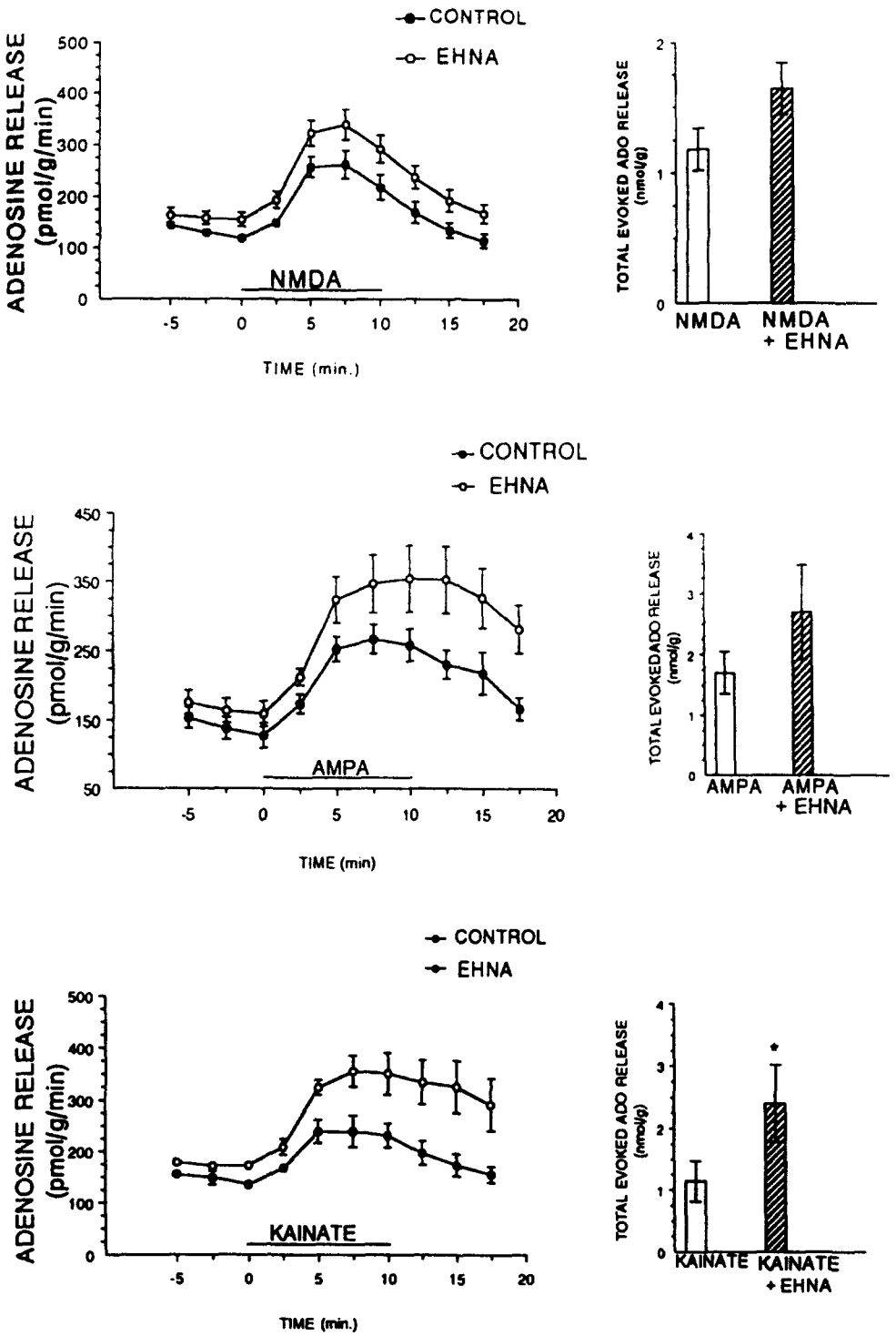

Fig. 4. Effect of inhibition of adenosine deaminase with a supramaximal concentration of EHNA (20 μM) on the formation of extracellular adenosine in rat cortical slices. Upper panel shows release of adenosine evoked by 100 μM NMDA ($n = 5$), middle panel shows release of adenosine evoked by 100 μM AMPA ($n = 8$), lower panel shows release of adenosine evoked by 100 μM kainate ($n = 3$). * Significantly different from control ($P < 0.05$, paired t test).

had relatively modest effects on adenosine release. EHNA (20 μM) slightly increased basal adenosine release 1.3-fold and increased the maximal rate of kainate-evoked adenosine release 1.5-fold. A very high concentration of 2′-deoxycoformycin (200 μM) increased basal adenosine release 1.6-fold, an effect similar to that reported previously for 2′-deoxycoformycin on basal release of adenosine in the striata of awake rats (Ballarin et al., 1991). 2′-Deoxycoformycin increased the maximal rates of evoked adenosine release 1.8-fold for NMDA, 1.7-fold for AMPA

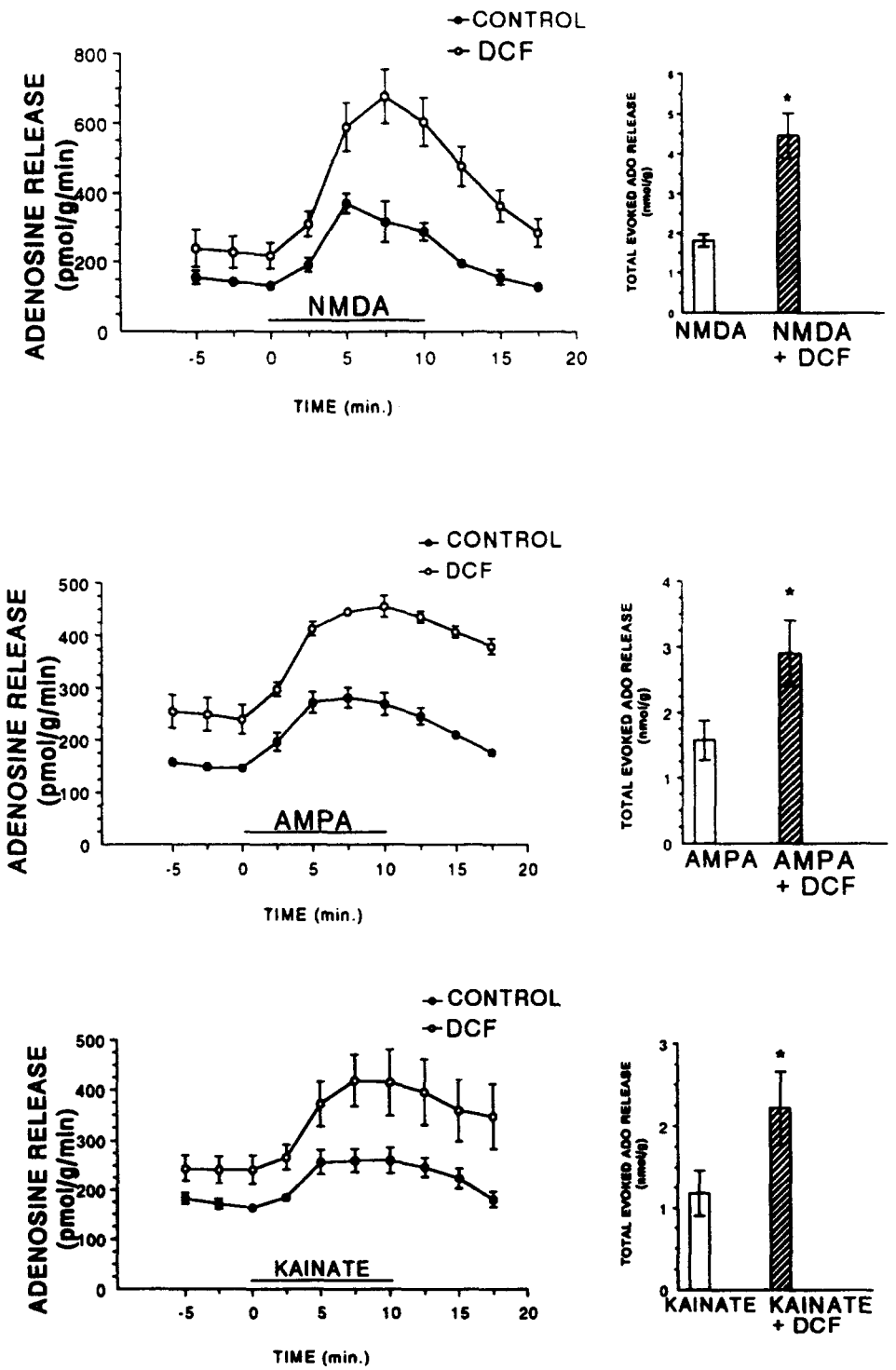

Fig. 5. Effect of inhibition of adenosine deaminase with a supramaximal concentration of 2′-deoxycoformycin (200 μM) on the formation of extracellular adenosine in rat cortical slices. Upper panel shows release of adenosine evoked by 100 μM NMDA ($n = 4$), middle panel shows release of adenosine evoked by 100 μM AMPA ($n = 3$), lower panel shows release of adenosine evoked by 100 μM kainate ($n = 3$). * Significantly different from control ($P < 0.05$, paired t test).

and 1.6-fold for kainate. The comparable effects of adenosine deaminase inhibitors on the rates of basal and EAA-evoked adenosine release observed in the present study suggest that this potentiation may not be as event-specific as that produced by adenosine kinase inhibtors. It also appears that adenosine deaminase inhibitors are considerably less effective than adenosine kinase inhibitors at potentiating the release of adenosine that occurs when

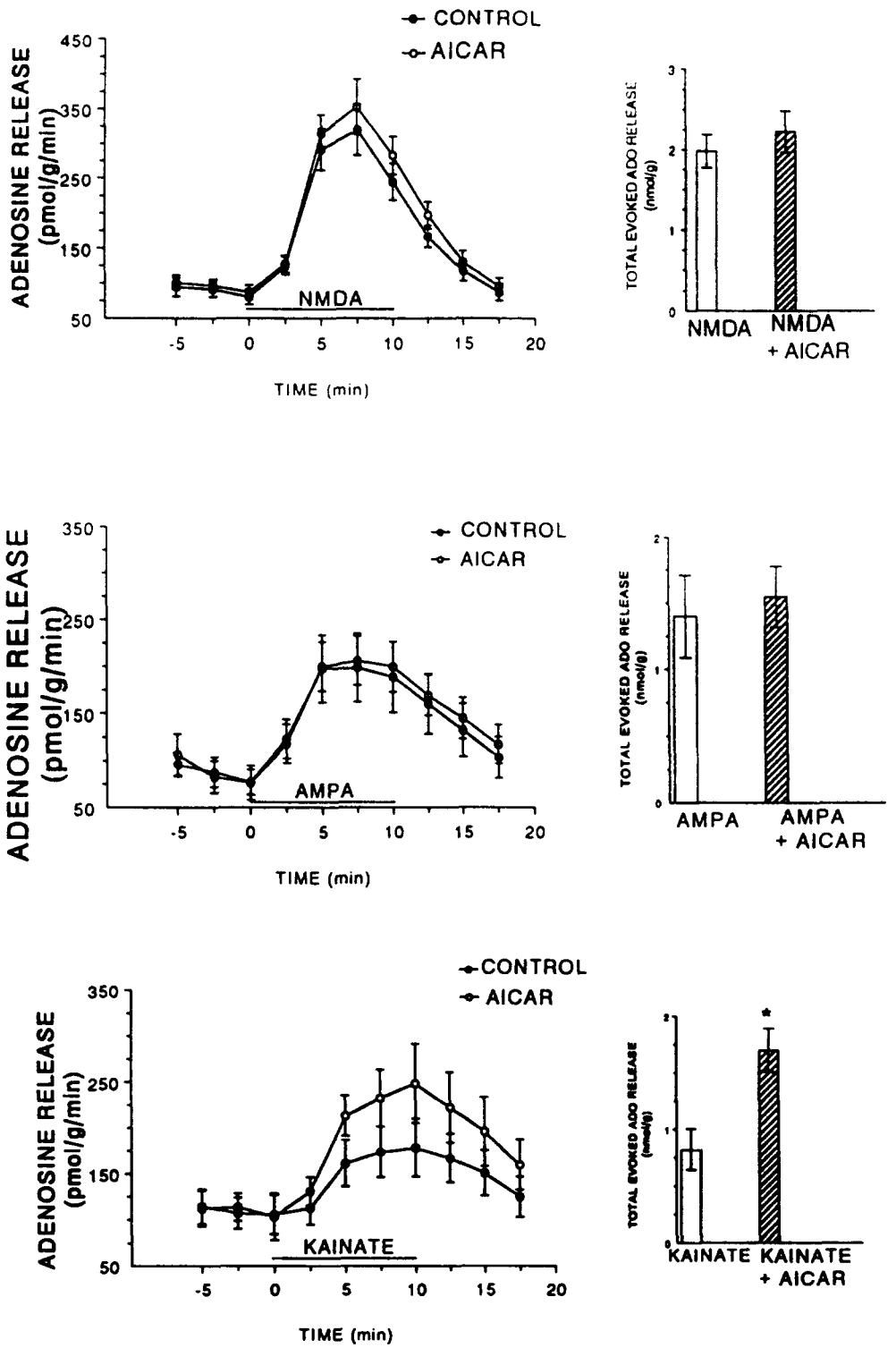

Fig. 6. Effect of AICAR (20 μM) on the formation of extracellular adenosine in rat cortical slices. Upper panel shows release of adenosine evoked by 100 μM NMDA ($n = 7$), middle panel shows release of adenosine evoked by 100 μM AMPA ($n = 5$), lower panel shows release of adenosine evoked by 100 μM kainate ($n = 4$). * Significantly different from control ($P < 0.05$, paired t test).

EAA receptors are activated, in so far as 20 μM aminodeoxyadenosine produced 3–4-fold increases in the total amount of adenosine released when EAA receptors were activated and 20 μM 5′-iodotubercidin produced 5–7-fold potentiations, whereas 20 μM EHNA only produced a 1.4-fold potentiation (kainate only) and 200 μM 2′-deoxycoformycin produced 2–2.5-fold potentiation. Moreover, at appropriate doses the adenosine kinase inhibitor 5′-iodotubercidin produced an increase in the basal rate of adenosine formation that was comparable to that observed with the adenosine deaminase inhibitor 2′-deoxycoformycin, but this level of adenosine kinase inhibition produced over twice as much potentiation of the maximal rate of NMDA-evoked adenosine formation than did inhibition of adenosine deaminase. These results suggest that adenosine kinase inhibition exhibits more event-specificity than does adenosine deaminase inhibition in potentiating extracellular adenosine formation, at least when the event is exposure to NMDA.

The results of other studies also suggest that adenosine kinase is an important regulator of extracellular adenosine levels in the brain. For instance, 5′-iodotubercidin appears to be more effective than EHNA at increasing extracellular adenosine levels in the caudate nuclei of anesthetized rats in vivo (Sciotti and Van Wylen, 1993). The authors concluded that extracellular adenosine levels are influenced to a greater extent by adenosine kinase than by adenosine deaminase. Pak et al. (1994) reported that inhibition of adenosine kinase with 5′-iodotubercidin suppressed population spikes in the CA1 pyramidal region of rat hippocampal slices, although no increases in endogenous adenosine release could be detected. However, the simultaneous inhibition of adenosine kinase and adenosine deaminase (with EHNA) caused a 3-fold increase in adenosine release from hippocampal slices. The authors concluded that adenosine kinase plays a significant role in regulating adenosine levels, and consequently synaptic transmission, in the hippocampus. Lloyd and Fredholm (1995) have also concluded that, under normoxic conditions, adenosine kinase plays a much greater role than adenosine deaminase in regulating both basal and electrically stimulated formation of extracellular adenosine in hippocampal slices. Although Mitchell et al. (1993) reported that inhibition of adenosine deaminase with EHNA enhanced both the duration and the amplitude of inhibition in the CA1 region of the hippocampus, Zhu and Krnjevic (1994) recently found no effect of adenosine deaminase inhibitors on transmission at CA1 in the hippocampus and concluded that adenosine deaminase does not appear to play a major role in controlling the levels of endogenous adenosine that modulate EAA receptor-mediated synaptic transmission in either normal or hypoxic hippocampal slices. On balance, these and the results of the present study support the conclusion that adenosine kinase plays a more significant role than adenosine deaminase in controlling extracellular adenosine levels in the brain.

AICAR (20 μM) had no effect on basal rates of adenosine release nor did it augment the maximal rates of NMDA- or AMPA-evoked adenosine release. However, AICAR did increase the maximal rate of kainate-evoked adenosine release 1.4-fold. The mechanism by which AICAR potentiates kainate-evoked adenosine release is not known but is unlikely to be due to inhibition of either adenosine kinase or adenosine deaminase because it is apparently a very weak inhibitor of these enzymes (Mullane, 1993). This very specific action of AICAR only on kainate-evoked adenosine release suggests that it (or similar agents) might be a useful and very selective therapeutic agent for treating disorders associated with excessive kainate receptor activation and resultant excitotoxicity, for example the excitotoxicity associated with the ingestion of mussels contaminated with the kainate agonist, domoic acid (Teitelbaum et al., 1990).

It is perhaps surprising that inhibition of adenosine kinase or adenosine deaminase had similar effects on both NMDA- and non-NMDA-evoked formation of extracellular adenosine since we showed previously that activation of the latter receptors releases adenosine itself whereas activation of NMDA receptors releases a nucleotide which is then degraded extracellularly to adenosine (Craig and White, 1993a). One might expect differential effects of these inhibitors on NMDA- vs. non-NMDA-evoked adenosine formation given these different sources of the nucleoside. Perhaps even more surprising is the distinct effect of AICAR on kainate- but not AMPA-evoked adenosine release, given the many similarities of their release profiles (Craig and White, 1993a). Explanations for these observations require further investigation.

The capacities of these drugs to potentiate the extracellular formation of adenosine appear to correlate well with their effectiveness as anticonvulsants. We showed previously that block of the nucleoside transporter with dipyridamole greatly potentiated the release of adenosine evoked by NMDA, kainate and AMPA (Craig and White, 1993a); although not shown in that paper, basal adenosine release was also increased substantially by dipyridamole, suggesting that dipyridamole may not exhibit event-specificity in augmentating adenosine release. The findings that block of the dipyridamole-sensitive adenosine transporter greatly potentiates EAA-evoked adenosine release from cortical slices (Craig and White, 1993a) and that adenosine kinase inhibitors are much more effective than adenosine deaminase inhibitors in augmenting EAA receptor-mediated adenosine release are completely consistent with the results of Zhang et al. (1993), where adenosine uptake blockers and adenosine kinase inhibitors were much more effective than adenosine deaminase inhibitors in preventing seizures in a bicuculline model of epilepsy. These authors also found that AICAR, which protects against ischemic myocardial damage by elevating extracellular adenosine (Mullane, 1993; Mullane and Young, 1993), did not exhibit antiseizure activity in their model. Signifi-

cantly, AICAR did not potentiate either the NMDA- or the AMPA-evoked formation of extracellular adenosine and only slightly potentiated kainate-evoked adenosine formation in the present study.

It appears that selectively potentiating the formation of extracellular adenosine that occurs when excitatory amino acid receptors are activated may be a useful approach in developing new therapies to treat seizure disorders and excitotoxicity. There is evidence that adenosine kinase inhibitors, such as the tubercidins and pyrazolo[3,4-*d*]pyrimidines, possess antiinflammatory actions resulting from increased extracellular adenosine which acts at A_2 receptors on neutrophils and diminishes their adherence, activation and phagocytosis (Cottam, 1994; Firestein et al., 1995). Since inflammation may contribute to neuronal degeneration in the CNS, inhibition of adenosine kinase would seem to be a particularly promising direction for developing new cerebroprotective therapies. Although inhibition of adenosine deaminase by itself is relatively ineffective in controlling seizures, Zhang et al. (1993) showed that the combination of an adenosine deaminase inhibitor with a marginally effective dose of an adenosine kinase inhibitor was fully efficacious. It seems possible that combinations of sub- or marginally effective doses of adenosine kinase, adenosine deaminase and/or adenosine uptake inhibitors might possess even more event-specificity in potentiating the EAA-evoked formation of extracellular adenosine and avoid some of the potential adverse effects of the individual drugs. This may be important when considering the potential clinical use of adenosine kinase inhibitors since adenosine kinase is found in all cells and may play an important role in maintaining adequate adenine nucleotide levels in various organs, including the heart (Kroll et al., 1993; Smolenski et al., 1994). We are currently investigating the effects that combinations of low doses of adenosine kinase inhibitors, adenosine deaminase inhibitors and adenosine uptake inhibitors have on the formation of extracellular adenosine evoked by EAAs.

We hope that these low-dose combinations of drugs may avoid some of the adverse side effects that the individual agents may possess.

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