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An adenosine A₃ receptor-selective agonist does not modulate calcium-activated potassium currents in hippocampal CA1 pyramidal neurons

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Introduction

The most recently discovered adenosine receptor, the A₃ receptor, belongs to the general class of G-protein coupled receptors, and has been linked to several putative effector mechanisms. One distinguishing characteristic of this receptor is that many adenosine agonists have affinities for the A₃ receptor that are much lower than their corresponding affinities at the adenosine A₁ receptor (Zhou et al., 1992), and this is true as well for the endogenous ligand, adenosine. It has been reported that A₃ receptors are coupled in an inhibitory fashion to adenylyl cyclase (Zhou et al., 1992; Zhao et al., 1997), although in other systems, A₃ receptor activation activates phospholipase C and leads to an elevation in inositol phosphate levels (Ali et al., 1990; Ramkumar et al., 1994; Auchampach et al., 1997), and this occurs in brain as well (Abbracchio et al., 1995). If this is the case, then increases in intracellular Ca²⁺ and activation of protein kinase C should occur as a consequence of agonist occupation of the A₃ receptor. Recent studies in brain have suggested that A₃ receptors can inhibit the function of presynaptic modulatory receptors (Dunwiddie et al., 1997; Macek et al., 1998), and that this effect is mediated via PKC (Macek et al., 1998; Diao and Dunwiddie, unpublished observations). However, other reports have proposed that postsynaptic A₃ receptors in hippocampus may elicit responses that are linked to activation of PKA but not PKC (Fleming and Mogul, 1996), suggesting that a further consideration of effector pathways for this receptor is warranted. One response in hippocampal CA1 pyramidal neurons that is affected by increases in intracellular Ca²⁺, as well as activation of either PKC or PKA, is the K⁺-mediated afterhyperpolarization that follows activation of voltage dependent Ca²⁺ channels by a depolarizing stimulus. Previous studies have demonstrated that activation of β-adrenergic receptors will nearly abolish the AHP (Madison and Nicoll, 1982; Haas and Konnerth, 1983), an effect that can be mimicked directly by activation of PKA (Abdul-Ghani et al., 1996). Activation of PKC with phorbol esters will also inhibit this response (Malenka et al., 1986), and activation of either muscarinic receptors (Cole and Nicoll, 1984) or metabotropic glutamate receptors (Abdul-Ghani et al., 1996) reduce the AHP via increases in intracellular Ca²⁺ and subsequent activation of CaM-K. Furthermore, adenosine itself has been previously reported to either increase (Haas and

Greene, 1984) or decrease (Dunwiddie, 1985) this current. Thus, this response appeared to be a likely candidate that would be sensitive to activation of multiple second messenger systems, but an effect of A₃ receptor activation on this conductance has not been described.

The recent development of CI-IB-MECA, an agonist that is approximately 2500-fold selective for the A₃ vs. the A₁ receptor, and 1400-fold selective for the A₃ vs. the A_{2a} receptor (Jacobson et al., 1995), has facilitated the study of the physiological consequences of selective activation of this receptor. Behavioral studies have demonstrated an A₃ receptor-mediated depression of locomotor activity (Jacobson et al., 1993), but the effects of A₃ receptor activation on neuronal activity at the cellular level are only poorly understood. A recent report has suggested that activation of A₃ receptors can increase Ca²⁺ currents in hippocampal CA3 neurons (Fleming and Mogul, 1996), but apart from this, there have been few reports of specific actions of A₃ receptor activation on neuronal activity.

Because the hippocampus and cerebellum show the highest levels of A₃ mRNA in brain (De et al., 1993), and because the responses to A₁ and A₂ receptor activation have been well-characterized in this brain region (Dunwiddie, 1985; Greene and Haas, 1991; Cunha et al., 1994, 1995), we have investigated the electrophysiological actions of CI-IB-MECA in this brain region.

Materials and methods

Slice preparation

Hippocampal slices were obtained from 6- to 8-week-old, male Sprague-Dawley rats (Sasco Animal Laboratories, Omaha, NE) using standard techniques (Dunwiddie and Lynch, 1978; Dunwiddie and Hoffer, 1980). Animals were decapitated, the hippocampus was dissected free of surrounding tissue, and 400 µm slices were cut from the middle portion of the hippocampus with a TC-2 tissue chopper (Sorvall, Norwalk, CT). Slices were immediately placed in ice-cold artificial cerebral spinal fluid (aCSF) consisting of (in mM): NaCl, 124; KCl, 3.3; KH₂PO₄, 1.2; MgSO₄, 2.4; CaCl₂, 2.5; D-glucose, 10; and NaHCO₃ 25.7, pH 7.4, pregassed with 95% O₂–5% CO₂, and were then transferred to an incubation chamber maintained at 33°C to equilibrate for at least 1 h before electrophysiological recording. When recording, the slices were transferred to a submersion recording chamber (1 ml volume) where they were placed on a nylon net and superfused with medium at a rate of 2 ml/min. The superfusion medium was gassed with humidified 95% O₂–5% CO₂ and maintained at a temperature of 33°C to 34°C.

Electrophysiological recording

Whole-cell patch recording experiments were performed using patch pipettes pulled from borosilicate glass (O.D. 1.5 mm, I.D. 0.86 mm, with filament; Sutter Instrument Co., Novato, CA). Electrodes had tip resistance of 5–8 MΩ when filled with a solution containing (in mM): K-Gluconate, 125; KCl, 15; HEPES, 10; CaCl₂, 0.1; KBAPTA, 1; K-ATP, 2; Tris-GTP, 0.3; pH adjusted to 7.3 with KOH, osmolarity adjusted to 280–290 mOsm. Whole-cell electrophysiological recordings were made using the “blind” patch recording technique (Blanton et al., 1989). The access resistance was continually monitored by observation of the cell membrane capacitive transients in response to a brief voltage step and was below 25 MΩ in all experiments. Pyramidal neurons were differentiated from glial cells by their ability to fire action potentials, and from interneurons by their high membrane capacitance and accommodation of cell firing in response to depolarizing current injection. Cells were voltages-clamped using an Axoclamp-2A amplifier (Axon-Instruments, Burlingame, CA) in the single-electrode voltage-clamp mode. The membrane potentials were corrected by – 11

mV for the liquid junction potential between the electrode filling solution and the bath (Neher, 1992).

I_{AHP} was evoked every 40 s by briefly (150 ms) stepping to 0 mV from holding potentials of -55 mV. Membrane resistance was determined by measuring the current change in response to a -4 mV command step every 40 s, and holding current was measured every 20 s. Responses were recorded using an R.C. Electronics ISC-16 A/D board and software developed in our laboratory.

At least 10 to 15 min of stable baseline responses were obtained in each experiment before drug applications began. Following acquisition of the baseline data, the effects of drugs were then tested by adding them directly to the superfusion medium with a calibrated syringe pump (Razel Scientific Instruments, Inc., Stamford, CT). The net change in flow rate using this approach was never more than 1%. The peak amplitude of the AHP current (I_{AHP}) was determined for each response and then averaged during the pre-drug control, drug, and the post-drug washout periods. At least 10 responses were included in each average. The data were analyzed as mean percent change in the response amplitude when compared to responses obtained during the control period.

Statistical analysis

All data were analyzed between groups, using the unpaired Student's *t*-test and nonparametric test (Mann-Whitney test) with a $P < 0.05$ criterion for statistical significance.

Chemicals

Adenosine, K-ATP, and Tris-ATP were obtained from Sigma (St. Louis, MO); baclofen, carbachol, 8-cyclopentyl-1,3-dimethylxanthine (CPT), and 3,7-dimethyl-1-propargylxanthine (DMPX) were purchased from Research Biochemicals (Natick, MA); 2-chloro- N^6 -(3-iodobenzyl)-adenosine-5-*N*-methyluronamide (Cl-IB-MECA) was provided by Research Biochemical International (Natick, MA) as part of the Chemical Synthesis Program of the National Institute of Mental Health, contract N01MH30003. The K-gluconate, KCl, $CaCl_2$ and HEPES used in the patch electrode recording solution were from Fluka Chemika-Biochemika (Ronkonkoma, New York), and K-BAPTA was from Molecular Probes (Eugene, Oregon). All drugs were dissolved in distilled water except CPT and Cl-IB-MECA, which were initially dissolved in 100% dimethylsulfoxide (DMSO) at greater than 1000 times final concentration and then diluted with aCSF to the desired concentration. The final concentration of DMSO in the superfusion medium never exceeded 0.05%.

Results

When hippocampal brain slices were superfused with adenosine ($50 \mu M$), a rapid outward current developed that showed little desensitization during a 10 min superfusion period, and reversed readily upon return to control buffer (Fig. 1A). This response was completely blocked by the selective adenosine A_1 receptor antagonist N^6 -cyclopentyl-theophylline (CPT; $1 \mu M$), and by CPT + DMPX (a moderately selective A_2 antagonist; $50 \mu M$). In either case, there was no significant effect of adenosine superfusion on the holding current (Fig. 1A). These latter conditions are nearly identical to those under which both adenosine and N^6 -2-(4-aminophenyl)ethyl-adenosine (APNEA) have been reported to increase Ca^{2+} currents in isolated hippocampal CA3 pyramidal neurons, an effect attributed to activation of A_3 receptors (Fleming and Mogul, 1997). These results suggested that under resting conditions, activation of A_3 receptors by adenosine did not have any significant effect upon the holding current in CA1 pyramidal neurons voltage clamped at -55 mV. This observation was confirmed by superfusion of slices with either 100 nM or $1 \mu M$ Cl-IB-MECA, also in

the presence of CPT + DMPX to block any possibility' of activation of A₁ or A₂ receptors; in this situation, there was also no significant change in the holding current required to clamp neurons to -55 mV (Fig. 1B).

It has been suggested that activation of protein kinase C (PKC) in hippocampal pyramidal neurons is linked to a reduction in the magnitude of afterhyperpolarizations (AHPs) induced by depolarizing current injection (Malenka et al., 1986; Engisch et al., 1996). Because previous studies have shown that A₃ receptors can also, be linked to activation of phospholipase C (PLC), and hence activation of PKC, we characterized the effects of adenosine and the selective A₃ agonist CI-IB-MECA on I_{AHP} . A 150 ms command step to 0 mV typically elicited a slow, outward current that lasted 10 s or more, and which was usually between 50–500 pA in amplitude (Fig. 2A₂, 2B₂). The time course of this response was consistent with the time course of the slow AHP activated by depolarizing current injection in current clamped neurons (Madison and Nicoll, 1982; Haas and Greene, 1984). To confirm the identity of this current, and to verify its previously reported sensitivity to other pharmacological agents, we examined the effects of carbachol (10 μ M; Fig. 2A), and isoproterenol (500 nM; Fig. 2B), both of which have been reported to inhibit this current. Bath superfusion with either of these agents virtually abolished the I_{AHP} , suggesting that under voltage clamp conditions, this current retained the pharmacological, sensitivity that has been previously described for the slow AHP.

In subsequent experiments, slices were super-fused with either 100 nM (Fig. 3A) or 1 μ M (Fig. 3B) CI-IB-MECA, and the amplitude of I_{AHP} was monitored. Neither concentration of this A₃ selective agonist had any significant effect on AHP amplitude. On the other hand, as we have previously noted (Dunwiddie, 1985), adenosine itself produced a rapid and highly significant reduction in the amplitude of I_{AHP} , and this effect reversed readily upon washing (Fig. 3C). The effect of adenosine, which was highly significant by itself ($t = 4.92$, $P < 0.01$), did not appear to be mediated via A₃ receptors, because this action was successfully antagonized by the combination of CPT + DMPX ($t = 1.66$, n.s.; Fig. 4A). On the other hand, neither concentration of CI-IB-MECA had a significant effect when tested on seven to 10 cells under the same conditions (Fig. 4B).

Thus, in summary, adenosine at a concentration of 50 μ M induced a significant outward change in the holding current, and significantly attenuated I_{AHP} , and both of these effects were completely blocked by the combination of the A₁ selective antagonist CPT and the somewhat A₂ selective antagonist DMPX (Fig. 5); these observations are consistent with previous work suggesting that A₁ receptor agonists can elicit both of these effects (Dunwiddie, 1985; Gerber et al., 1989). On the other hand, CI-IB-MECA had no significant effect on either one of these parameters, and this was true both when it was tested alone, and in the presence of CPT + DMPX (Fig. 5).

Discussion

The slow AHP that can be elicited in hippocampal pyramidal neurons by brief depolarization is a response that has been shown to be quite sensitive to activation of multiple second messenger systems, and appears to be inhibited by activation of PKA, PKC, and CaM-K. For these reasons, it appeared to be an appropriate candidate to determine whether activation of A₃ receptors was linked to changes in the activity of any of these kinases. However, the results of these studies demonstrated that neither the selective A₃ receptor agonist, CI-IB-MECA, nor the non-selective ligand adenosine (in the presence of A₁- and A₂-selective blockers), had any significant effect on the amplitude of I_{AHP} . Furthermore, these results also suggest that A₃ receptor activation does not inhibit the voltage gated Ca²⁺ channels that mediate the Ca²⁺ influx that activates this conductance. In

the absence of antagonists, adenosine elicited a reduction in the amplitude of I_{AHP} , but these effects were blocked by the combination of the A_1 (CPT) and A_2 (DMPX) selective antagonists. This is most probably the result of a direct reduction in Ca^{2+} channel activity mediated via A_1 receptors, or the activation of inwardly rectifying K^+ channels via A_1 receptors, which could indirectly reduce the Ca^{2+} influx. In an intact system such as the brain slice, it is not possible to effectively clamp CA1 pyramidal neurons during a depolarizing step to 0 mV; the effectiveness of the clamp, and hence the net Ca^{2+} influx, can be affected by the activation of other conductances, such as the inwardly-rectifying K^+ channels that are linked to activation of A_1 receptors.

At least three kinds of responses might have been expected based upon the known actions of A_3 receptors. First, the enhancement of Ca^{2+} currents that has been associated with A_3 receptor activation in CA3 neurons (Fleming and Mogul, 1997) might have been expected to lead to an enhancement of I_{AHP} . Second, activation of PLC would have been expected to lead to a reduction in I_{AHP} , either via activation of PKC, or via the IP3-dependent release of intracellular Ca^{2+} . Finally, because it has been proposed that A_3 receptors may activate PKA in hippocampal neurons (Fleming and Mogul, 1997), this might also have been expected to lead to a decrease in I_{AHP} .

The fact that none of these effects were observed suggests several possibilities. It is possible that there were offsetting effects, such that an enhancement of Ca^{2+} influx combined with second messenger mediated down-regulation of the I_{AHP} led to no significant change in the current. Although possible, it seems unlikely that such precisely offsetting responses could occur in every slice; the lack of change even in the variability of the response (Fig. 4B) is not consistent with this conclusion. A somewhat more likely alternative perhaps is that the effects on Ca^{2+} currents are confined solely to CA3 neurons; little work has been done on the localization of A_3 receptors, and it is certainly possible that the CA1 and CA3 neurons differ in terms of receptor expression and/or coupling. Alternatively, A_3 receptors may be present on CA1 pyramidal neurons, but localized in such a way that they do not directly affect I_{AHP} . Much recent work on adenylyl cyclase has emphasized the localization or anchoring of this enzyme near appropriate substrates, and it is possible that while β -receptors activate adenylyl cyclase in a subcellular compartment with ready access to the channels that mediate I_{AHP} , A_3 receptors activate a cyclase that lacks such access. Future experiments will be required to determine which of these alternatives is the case.

Conclusions

The adenosine A_3 receptor is found in brain, and has been linked to a number of physiological responses, but the cellular mechanisms that underlie these effects are still unclear. In the present experiments, the effects of a selective A_3 agonist, 2 - chloro - N^6 - (3 - iodobenzyl) - adenosine - 5' - N - methyluronamide (CI-IB-MECA), were characterized on hippocampal CA1 pyramidal neurons. More specifically, we examined the possibility that activation of A_3 receptors could lead to activation of protein kinase A (PKA), protein kinase C (PKC), or calcium/calmodulin dependent protein kinase II (CaM-K), which might then induce significant changes in the activity of hippocampal pyramidal neurons. CA1 pyramidal neurons were recorded in the whole cell mode using patch electrodes, and the effects of adenosine agonists were determined on the holding current, and on the amplitude of the slow outward calcium-activated K^+ currents activated following a depolarizing step in the command voltage (I_{AHP}). As has been previously reported, a variety of agents reduced the I_{AHP} , including the (β -adrenoceptor agonist isoproterenol, the muscarinic agonist carbachol, and adenosine itself; however, CI-IB-MECA had no significant effects on this current. CI-IB-MECA by itself also had no effect on the holding current, and had a weak but not statistically significant antagonistic effect on the outward currents induced by activation

of A₁ receptors on pyramidal neurons. These results suggest that either A₃ receptors are not present on the cell bodies of hippocampal CA1 pyramidal neurons, that they are present but not linked to PKA, PKC, or CaM-K activation, or that they only activate these kinases in cell compartments that are not accessible to the ion channels that underlie the AHP response.

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References

- Abbracchio MP, Brambilla R, Ceruti S, Kim HO, von Lubitz DKJE, Jacobson KA, Cattabeni F. G protein-dependent activation of phospholipase C by adenosine A₃ receptors in rat brain. *Mol Pharmacol*. 1995; 48:1038–1045. [PubMed: 8848003]
- Abdul-Ghani MA, Valiante TA, Carlen PL, Pennefather PS. Metabotropic glutamate receptors coupled to IP₃ production mediate inhibition of IAHP in rat dentate granule neurons. *J Neurophys*. 1996; 76:2691–2700.
- Ali H, Cunha-Melo JR, Saul WF, Beaven MA. Activation of phospholipase C via adenosine receptors provides synergistic signals for secretion in antigen-stimulated RBL-2H3 cells. Evidence for a novel adenosine receptor. *J Biol Chem*. 1990; 265:745–753. [PubMed: 2295618]
- Auchampach JA, Rizvi A, Qiu Y, Tang XL, Maldonado C, Teschner S, Bolli R. Selective activation of A₃ adenosine receptors with N⁶-(3-iodobenzyl)adenosine-5'-N-methyluronamide protects against myocardial stunning and infarction without hemodynamic changes in conscious rabbits. *Circ Res*. 1997; 80:800–809. [PubMed: 9168782]
- Blanton MG, Lo Turco JJ, Kriegstein AR. Whole cell recording from neurons in slices of reptilian and mammalian cerebral cortex. *J Neurosci Meth*. 1989; 30:203–210.
- Bruns RF, Daly JW, Pugsley TA. Characterization of A₂ receptor labeled by [³H]-NECA in rat striatal membranes. *Mol Pharmacol*. 1986; 29:331–346. [PubMed: 3010074]
- Cole AE, Nicoll RA. Characterization of a slow cholinergic post-synaptic potential recorded in vitro from rat hippocampal pyramidal cells. *J Physiol (Lond)*. 1984; 352:173–188. [PubMed: 6747887]
- Cunha RA, Johansson B, Fredholm BB, Ribeiro JA, Sebastião AM. Adenosine A_{2A} receptors stimulate acetylcholine release from nerve terminals of the rat hippocampus. *Neurosci Lett*. 1995; 196:41–44. [PubMed: 7501252]
- Cunha RA, Johansson B, Van der Ploeg I, Sebastião AM, Ribeiro JA, Fredholm BB. Evidence for functionally important adenosine A_{2A} receptors in the rat hippocampus. *Brain Res*. 1994; 649:208–216. [PubMed: 7953635]
- De M, Austin KF, Dudley MW. Differential distribution of A₃ receptor in rat brain. *Soc Neurosci Abstr*. 1993; 19:42.11. (Abstract).
- Dunwiddie TV. The physiological role of adenosine in the central nervous system. *Int Rev Neurobiol*. 1985; 27:63–139. [PubMed: 2867982]
- Dunwiddie TV, Diao LH, Kim HO, Jiang JL, Jacobson KA. Activation of hippocampal adenosine A₃ receptors produces a desensitization of A₁ receptor-mediated responses in rat hippocampus. *J Neurosci*. 1997; 17:607–614. [PubMed: 8987783]
- Dunwiddie TV, Hoffer BJ. Adenine nucleotides and synaptic transmission in the in vitro rat hippocampus. *Br J Pharmacol*. 1980; 69:59–68. [PubMed: 6247005]
- Dunwiddie TV, Lynch GS. Long-term potentiation and depression of synaptic responses in the rat hippocampus: Localization and frequency dependency. *J Physiol (Lond)*. 1978; 276:353–367. [PubMed: 650459]
- Engisch KL, Wagner JJ, Alger BE. Whole-cell voltage-clamp investigation of the role of PKC in muscarinic inhibition of IAHP in rat CA1 hippocampal neurons. *Hippocampus*. 1996; 6:183–191. [PubMed: 8797018]
- Fleming KM, Mogul DJ. Adenosine A₃ receptors potentiate hippocampal calcium current by a PKA-dependent/PKC-independent pathway. *Drug Dev Res*. 1996; 37:121. (Abstract).

- Fleming KM, Mogul DJ. Adenosine A₃ receptors potentiate hippocampal calcium current by a PKA-dependent/PKC-independent pathway. *Neuropharmacol.* 1997; 36:353–362.
- Gerber U, Greene RW, Haas HL, Stevens DR. Characterization of inhibition mediated by adenosine in the hippocampus of the rat in vitro. *J Physiol (Lond).* 1989; 417:567–578. [PubMed: 2559967]
- Greene RW, Haas HL. The electrophysiology of adenosine in the mammalian central nervous system. *Prog Neurobiol.* 1991; 36:329–341. [PubMed: 1678539]
- Haas HL, Greene RW. Adenosine enhances afterhyperpolarization and accommodation in hippocampal pyramidal cells. *Pflugers Arch.* 1984; 402:244–247. [PubMed: 6097865]
- Haas HL, Konnerth A. Histamine and noradrenaline decrease calcium-activated potassium conductance in hippocampal pyramidal cells. *Nature.* 1983; 302:432–434. [PubMed: 6300681]
- Jacobson KA, Kim HO, Siddiqi SM, Olah ME, Stiles GL, von Lubitz DKJE. A₃-adenosine receptors: design of selective ligands and therapeutic prospects. *Drugs of the Future.* 1995; 20:689–699.
- Jacobson KA, Nikodijevic O, Shi D, Gallo-Rodriguez C, Olah ME, Stiles GL, Daly JW. A role for central A₃ adenosine receptors: Mediation of behavioral depressant effects. *FEBS Lett.* 1993; 336:57–60. [PubMed: 8262217]
- Macek TA, Schaffhauser H, Conn PJ. Protein kinase C and A₃ adenosine receptor activation inhibit presynaptic metabotropic glutamate receptor (mGluR) function and uncouple mGluRs from GTP-binding proteins. *J Neurosci.* 1998; 18:6138–6146. [PubMed: 9698308]
- Madison DV, Nicoll RA. Noradrenaline blocks accommodation of pyramidal cell discharge in the hippocampus. *Nature.* 1982; 299:636–638. [PubMed: 6289127]
- Malenka RC, Madison DV, Andrade R, Nicoll RA. Phorbol esters mimic some cholinergic actions in hippocampal pyramidal neurons. *J Neurosci.* 1986; 6:475–480. [PubMed: 3456434]
- Neher E. Ion channels for communication between and within cells. *Science.* 1992; 256:498–502. [PubMed: 1373906]
- Ramkumar V, Stiles GL, Beaven MA, Ali H. The A₃ adenosine receptor is the unique adenosine receptor which facilitates release of allergic mediators in mast cells. *J Biol Chem.* 1994; 268:16887–16890. [PubMed: 8349579]
- Seale TW, Abila KA, Shamim MT, Carney JM, Daly JW. 3,7-Dimethyl-1-propargylxanthine: A potent and selective in vivo antagonist of adenosine analogs. *Life Sci.* 1988; 43:1671–1684. [PubMed: 3193854]
- Ukena D, Shamim MT, Padgett W, Daly JW. Analogs of caffeine: Antagonists with selectivity for A₂ adenosine receptors. *Life Sci.* 1986; 39:743–750. [PubMed: 3016449]
- Zhao ZH, Francis CE, Ravid K. An A₃-subtype adenosine receptor is highly expressed in rat vascular smooth muscle cells: Its role in attenuating adenosine-induced increase in cAMP. *Microvasc Res.* 1997; 54:243–252. [PubMed: 9441895]
- Zhou QY, Li C, Olah ME, Johnson RA, Stiles GL, Civelli O. Molecular cloning and characterization of an adenosine receptor: The A₃ adenosine receptor. *Proc Natl Acad Sci (USA).* 1992; 89:7432–7436. [PubMed: 1323836]

Fig. 1.

Effect of adenosine and Cl-IB-MECA on holding currents. Changes induced in current required to clamp CA1 hippocampal pyramidal neurons to -55 mV are indicated for groups of cells treated with identical drug superfusion protocols. Although not indicated, CPT + DMPX superfusion was maintained for at least 20 min prior to superfusion with adenosine or Cl-IB-MECA. CPT is an A_1 selective antagonist with approximately 130X selectivity for A_1 vs. A_{2A} receptors and a K_1 for A_1 receptors of 11 nM (Bruns et al., 1986); DMPX has a K_1 of 11 μ M at A_{2A} receptors and is 4–11 x selective for A_{2A} vs. A_1 (Ukena et al., 1986; Seale et al., 1988). Adenosine induced a highly significant outward change in the holding current (A) and this effect was blocked by CPT + DMPX. Neither concentration of Cl-IB-MECA produced any change in the holding current in the presence of the A_1/A_2 receptor antagonists.

Fig. 2.

Effects of carbachol and isoproterenol on I_{AHP} . Superfusion with 10 μM carbachol (A₁) or with 500 nM isoproterenol (ISO; B₁) rapidly blocked the currents evoked by a 150 ms depolarizing step to 0 mV. The amplitudes shown at the left correspond to the peak amplitude of the I_{AHP} , or in the case of the inhibited responses, to the holding current at the time corresponding to the peak amplitude of I_{AHP} prior to drug superfusion. The actual currents elicited by the depolarizing step are indicated at right (A₂ and B₂); the numbers in the figures at the left indicate the times at which the actual waveforms at the right were acquired.

Fig. 3.

Effects of CI-IB-MECA and adenosine on I_{AHP} amplitude. The time courses of changes in the amplitude of I_{AHP} are shown at the left and averaged responses obtained at the indicated times at the right, as in Fig. 2. Neither concentration of CI-IB-MECA had any significant effect on the response, but 50 μ M adenosine depressed I_{AHP} amplitude in a readily reversible fashion.

Fig. 4.

Average time course of the effects of Cl-IB-MECA and adenosine on I_{AHP} amplitude. Groups of slices were superfused with drugs using identical treatment protocols, and the mean $\pm$ SEM response as a percentage of pre-drug response amplitude is illustrated. Adenosine induced a reversible depression of this response that was blocked by CPT + DMPX (A), whereas Cl-IB-MECA had no effect, either in the absence of inhibitors (Fig. 3A and B), or in slices pretreated with the A_1/A_2 inhibitors (B).

Fig. 5.

Average effects of drug superfusion on holding current and I_{AHP} . The mean $\pm$ SEM response for changes in the holding current (upper) and I_{AHP} (lower) are shown. All of the effects observed with adenosine were completely antagonized by CPT + DMPX (** $P < 0.01$; hatched bars), whereas CI-IB-MECA had no significant effect upon either measure, either alone or in combination with the combined antagonists.