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Tonic activation of A_{2A} adenosine receptors unmasks, and of A_1 receptors prevents, a facilitatory action of calcitonin gene-related peptide in the rat hippocampus

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1 We investigated how manipulations of the degree of activation of adenosine A_1 and A_{2A} receptors influences the action of the neuropeptide, calcitonin gene-related peptide (CGRP) on synaptic transmission in hippocampal slices. Field excitatory post-synaptic potentials (EPSPs) from the CA1 area were recorded.

2 When applied alone, CGRP (1–30 nM) was without effect on field EPSPs. However, CGRP (10–30 nM) significantly increased the field EPSP slope when applied to hippocampal slices in the presence of the A_1 receptor antagonist, 1,3-dipropyl-8-cyclopenthyl xanthine (DPCPX, 10 nM), or in the presence of the A_{2A} adenosine receptor agonist CGS 21680 (10 nM).

3 The A_{2A} receptor antagonist, ZM 241385 (10 nM) as well as adenosine deaminase (ADA, 2 U ml^{-1}), prevented the enhancement of field EPSP slope caused by CGRP (30 nM) in the presence of DPCPX (10 nM), suggesting that this effect of CGRP requires the concomitant activation of A_{2A} adenosine receptors by endogenous adenosine.

4 The protein kinase-A inhibitors, *N*-(2-guanidinoethyl)-5-isoquinolinesulfonamide (HA-1004, 10 μM) and adenosine 3′,5′-cyclic monophosphorothioate, Rp-isomer (Rp-cAMPS, 50 μM), as well as the inhibitor of ATP-sensitive potassium (K_{ATP}) channels, glibenclamide (30 μM), prevented the facilitation of synaptic transmission caused by CGRP (30 nM) in the presence of DPCPX (10 nM), suggesting that this effect of CGRP involves both K_{ATP} channels and protein kinase-A.

5 It is concluded that the ability of CGRP to facilitate synaptic transmission in the CA1 area of the hippocampus is under tight control by adenosine, with tonic A_1 receptor activation by endogenous adenosine 'braking' the action of CGRP, and the A_{2A} receptors triggering this action. *British Journal of Pharmacology* (2000) **129,** 374–380

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Abbreviations: ADA, adenosine deaminase; CGRP, calcitonin gene-related peptide; CGS 21680, 2-[p-(2-carboxyethylphenethylamino]-5′-N-ethylcarboxamide adenosine; DPCPX, 1,3-dipropyl-8-cyclopenthyl xanthine; EPSP, excitatory postsynaptic potential; HA-1004, *N*-(2-guanidinoethyl)-5-isoquinolinesulfonamide (HA-1004); K_{ATP} channels, ATP-sensitive potassium channels; PK-A, protein kinase-A; Rp-cAMPS, adenosine 3′,5′-cyclic monophosphorothioate; ZM 241385, (4-(2-[7-amino-2-(2-furyl)[1,2,4]triazolo[2,3-a][1,3,5]triazin-5ylamino]ethyl) phenol)

Introduction

Adenosine, by operating inhibitory A_1 receptors or excitatory A_2 receptors, has widespread modulatory actions in the nervous system (for a review see Sebastião & Ribeiro, 1996) and may interfere with the action of other neuromodulator/neurotransmitter substances (Ribeiro, 1999). Of particular interest in the context of the present study are the evidences that A_{2A} receptor activation inhibits A_1 receptor function in the hippocampus (Cunha *et al*., 1994) and that tonic activation of adenosine A_{2A} receptors facilitates the action of other neuromodulators, such as that of the neuropeptide calcitonin gene-related peptide (CGRP) at motor nerve terminals (Correia-de-Sá & Ribeiro, 1994). Adenosine, through A_1 receptor activation, may also interfere with CGRP release (Santicioli *et al*., 1993).

CGRP is a 37-amino acid peptide generated from the calcitonin gene by alternate tissue-specific splicing (Amara *et al*., 1982). It is widely present in the peripheral and in the central nervous system (for reviews see Ishida-Yamamoto & Tohyama, 1989; Poyner, 1992), including the hippocampus (Bulloch *et al*., 1996; Freund *et al*., 1997; Oliver *et al*., 1999), where it inhibits NMDA receptor-mediated actions (Bouchard *et al*., 1995) and may protect against injury (Bulloch *et al*., 1996; 1998).

The present work was designed to investigate how manipulations of the degree of activation of adenosine A_1 and A_{2A} receptors influences the action of CGRP on synaptic transmission in the hippocampus. Since the predominant action of endogenous adenosine on synaptic transmission in the hippocampus is inhibitory, through A_1 receptor activation (e.g. Dunwiddie & Diao, 1994), we first evaluated how blockade of A_1 receptors could influence the action of CGRP. Because synaptic transmission in the hippocampus can be influenced by both inhibitory A_1 (Sebastião *et al*., 1990) and excitatory A_{2A} (Sebastião & Ribeiro, 1992) adenosine receptors, we then investigated how the simultaneous blockade of these two adenosine receptor subtypes, and how removal of endogenous extracellular adenosine with adenosine deaminase, could influence the effect of CGRP. How A_{2A} adenosine receptor activation by a selective agonist interferes with the action of CGRP on hippocampal synaptic transmission, was

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also evaluated. Since several CGRP-mediated action involve activation of protein-kinase A and of ATP-sensitive potassium channels (K_{ATP} channels) (e.g. Zang *et al*., 1994; Santicioli & Maggi, 1994; Wellman *et al*., 1998), we investigated how inhibition of PK-A with HA-1004 (Hidaka & Kobayahi, 1992) or with the cyclic AMP analogue, RpcAMPS (Rothermel & Parker-Bothelho, 1998) and how inhibition of K_{ATP} channels with glibenclamide (Schmid-Antomarchi *et al*., 1987) affects the action of CGRP on synaptic transmission in the hippocampus.

A preliminary account of this study has been presented to the 6th International Symposium of Adenosine and Adenine Nucleotides, in Ferrara, Italy, May 1998 (Sebastião *et al*., 1998) and to the British Pharmacological Society (Sebastião *et al*., 1999)

Methods

The experiments were performed on hippocampal slice preparations taken from male Wistar rats (5–6 weeks old) handled according to the European Community guidelines on Animal Care. The animals were decapitated under halothane anaesthesia and the hippocampus dissected free into ice-cold Krebs solution of the following composition (mM): NaCl 124, KCl 3, NaH_2PO_4 1.25, $NaHCO_3$ 26, $MgSO_4$ 1, $CaCl_2$ 2, glucose 10, previously gassed with 95% O_2/5% CO_2 (pH ≈ 7.4). Slices (400 μm thick) were cut perpendicular to the long axis of the hippocampus with a McIlwain tissue chopper, and allowed to recover for at least 1 h in a chamber within the same gassed medium at room temperature (22–25°C). A slice was then transferred to a 1 ml (plus 2 ml dead volume) recording chamber for submerged slices and continuously superfused with gassed bathing solution at 30°C, at a flow rate of 3 ml min^{-1}. Drugs were added to this superfusion solution.

Monopolar stimulation (rectangular pulse of 0.1 ms applied once every 10 s) was delivered through a concentric electrode placed on the Schaffer collateral/commissural fibres, in the stratum radium near the CA3/CA1 border. Evoked field excitatory post-synaptic potentials (field EPSPs) were recorded through an extracellular electrode (4 M NaCl, 3–5 MΩ resistance) placed in the stratum radiatum of the CA1 area. The intensity of the stimulus (80–400 μA) was initially adjusted to obtain a large field EPSP slope with a minimum population spike contamination. Recordings were obtained with an Axoclamp 2B amplifier coupled to a DigiData 1200 interface (Axon Instruments). Averages of eight consecutive responses were continuously monitored on a personal computer with the LTP program (Anderson & Collingridge, 1997), kindly supplied by W.W. Anderson (University of Bristol, U.K.). Responses were quantified as the slope of the initial phase of the averaged field EPSPs, since slope measures are considered a more accurate measure of field EPSP magnitude than the amplitude, due to contamination by population spike.

To minimise underestimation of the CGRP responses due to desensitization (see Aiyar *et al*., 1992), only one concentration of CGRP was applied to each slice, and thus, the concentration response curves showed in this paper were performed in a non-cumulative manner. CGRP was applied to each slice either alone or in the presence of adenosine receptor agonists and/or antagonists. Whenever the effect of CGRP was tested in the presence of other drugs, the neuropeptide was applied to the preparations only after a stable response to these drugs was observed and at least 20 min after starting their perfusion. Because CGRP is readily adsorbed onto plastic and glass, the chamber and all the connecting tubes were pre-treated with Sigmacote to minimise losses of the peptide (see Afonso *et al*., 1996).

Drugs

Rat calcitonin gene-related peptide (CGRP) and sigmacote were from Sigma. CGS 21680 (2-[p-(2-carboxyethylphenethylamino]-5′-N-ethylcarboxamide adenosine) and 1,3-dipropyl-8-cyclopentylxanthine (DPCPX) were from Research Biochemicals Inc. (R.B.I.). ZM 241385 (4-(2-[7-amino-2-(2-furyl)[1,2,4]-triazolo[2,3-a][1,3,5]triazin-5ylamino]ethyl)phenol), was a kind gift from ZENECA. DPCPX was made up in a 5 mM stock solution in 99% dimethylsuphoxide (DMSO)/1% NaOH1 M (v v^{-1}). CGS 21680 and ZM 241385 was made up in 5 mM stock solutions in DMSO. CGRP was made up as a 1 mM stock solution in distilled water. Aliquots of the stock solutions were kept frozen at −20°C until use. In each experiment, one aliquot was thawed and diluted in Krebs solution.

Analysis of the data

The data are expressed as means ± s.e.mean from n number of slices. Unless otherwise stated, the significance of the differences between the means was evaluated by the Student's t-test. When comparing more than two experimental groups (Figure 3), one-way repeated measures ANOVA was used followed by the Newman-Keuls test. Values of $P<0.05$ were considered to represent statistically significant differences.

Results

Influence of A_1 receptor blockade upon the effect of calcitonin gene-related peptide (CGRP) on hippocampal synaptic transmission

To prevent A_1 receptor activation by endogenous adenosine, we used the A_1 receptor antagonist, 1,3-dipropyl-8-cyclopentylxanthine (DPCPX) at a concentration (10 nM) 20 times higher its affinity value for the A_1 receptors in the hippocampus (see Sebastião *et al*., 1990). At this concentration, DPCPX increased the slope of the field EPSPs by 21 ± 4.2% ($n=20$, $P<0.05$), which is consistent with its ability to prevent the tonic inhibition of synaptic transmission caused by endogenous adenosine.

When applied to hippocampal slices in the absence of DPCPX, CGRP in low nanomolar concentrations (1–30 nM) was virtually devoid of effect on the field EPSPs. However, as illustrated in Figure 1, when CGRP (30 nM) was applied to an hippocampal slice after a stable response to DPCPX was recorded, the neuropeptide caused a consistent and reversible increase (17 ± 1.3%, $n=19$, $P<0.05$) in the slope of the field EPSPs. The maximal effect of the neuropeptide was obtained 15–20 min after starting CGRP perfusion and was reversed within 15–20 min after stopping CGRP perfusion (Figure 1).

In Figure 2 are compared the concentration-response curves for the effects of CGRP on the slope of field EPSPs recorded in the absence and in the presence of DPCPX (10 nM). In the presence of this A_1 receptor antagonist, but not in its absence, CGRP concentration-dependently increased the slope of the field EPSPs when tested in concentrations up to 30 nM. At an higher concentration (100 nM) CGRP inhibited the slope of the field EPSPs by 15 ± 6.2% ($n=3$), an action virtually not modified when A_1 receptors were blocked by DPCPX (10 nM), the average

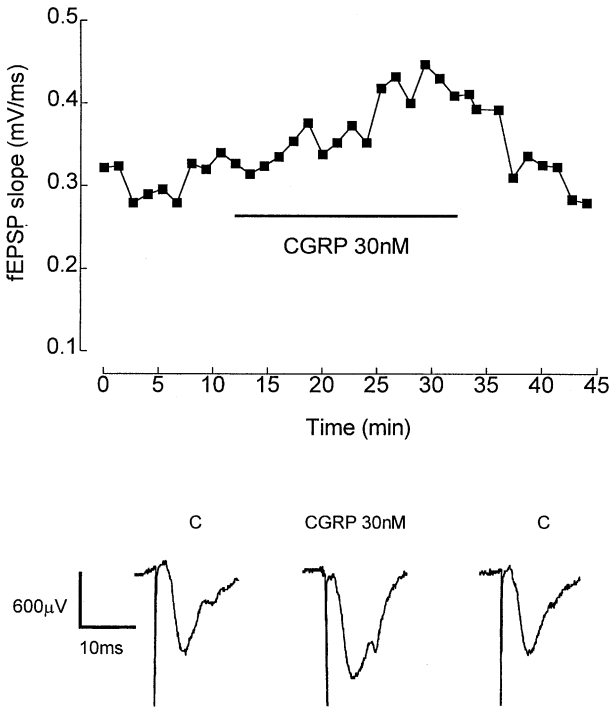

Figure 1 Enhancement caused by calcitonin gene-related peptide (CGRP, 30 nM) on the slope of field excitatory postsynaptic potentials (fEPSPs) recorded from the CA1 area of an hippocampal slice in the presence of the A_1 adenosine receptor antagonist, 1,3-dipropyl-8-cyclopentylxanthine (DPCPX, 10 nM). The upper panel shows the time course of the effect of CGRP. Each point, in the ordinates, corresponds to the slope of the average of eight consecutive field EPSPs, and in the abscissae to the start of averaging. The time of perfusion of CGRP is indicated by the horizontal bar, and DPCPX (10 nM) was always present. The lower panel shows recording of field EPSPs (each an average of eight consecutive responses) obtained (from left to right): before perfusion of CGRP, at the maximum effect of CGRP (30 nM), and after washing out CGRP; the field EPSPs are preceded by the synaptic volley and the stimulus artefact.

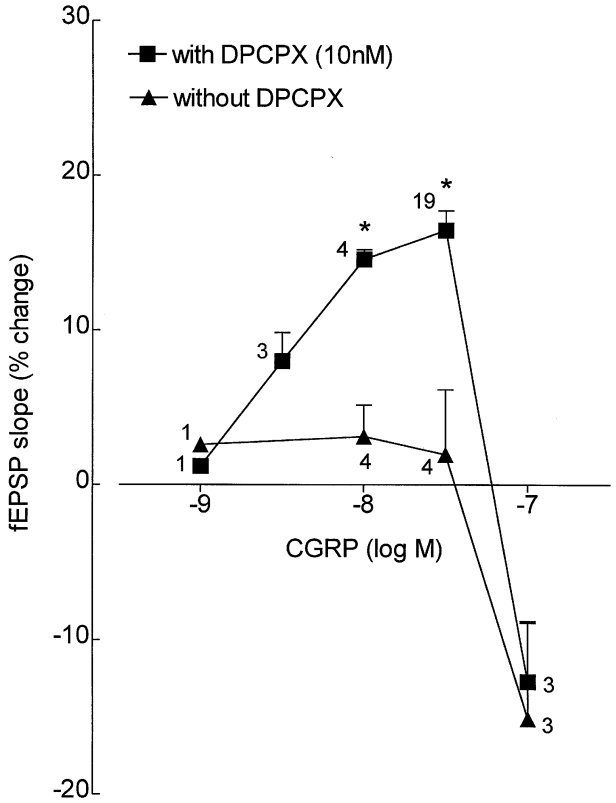

Figure 2 Concentration-response curves for the effects of calcitonin gene-related peptide (CGRP) on the slope of field excitatory postsynaptic potentials (fEPSPs) recorded from the CA1 area of the rat hippocampal slices in the absence and in the presence of the A_1 adenosine receptor antagonist, 1,3-dipropyl-8-cyclopentylxanthine (DPCPX), as indicated by the symbols. Each point represents the mean ± s.e.mean results obtained in *n* slices, as indicated close to each symbol. The concentration-response curves were obtained in a non-cumulative way, and in each slice CGRP was applied only once. The field EPSP slope before CGRP (0%) was 0.41 ± 0.05 mV ms^{-1} ($n=12$) in the experiments without DPCPX and 0.48 ± 0.03 mV ms^{-1} ($n=30$) in the experiments with DPCPX. *$P<0.05$ (Student's *t*-test) as compared with the effect of CGRP in the absence of DPCPX.

inhibition caused by CGRP under this condition being $13 \pm 3.9\%$ ($n=3$) (Figure 2).

Influence of combined A_1 and A_{2A} adenosine receptors blockade, and of removal of endogenous adenosine, on the effect of CGRP on synaptic transmission

In a first set of experiments we applied CGRP to hippocampal slices where A_1 and A_{2A} adenosine receptors have been blocked. To antagonize A_{2A} receptors we used the selective antagonist, ZM 241385, which has been shown to prevent adenosine A_{2A} receptor-mediated actions in the hippocampus (Cunha *et al.*, 1997); CGRP was tested in the concentration (30 nM) that caused maximal enhancement of the field EPSPs in the presence of DPCPX (10 nM). As illustrated in Figure 3A, CGRP (30 nM) was virtually devoid of effect on the field EPSPs when applied to slices where both A_1 and A_{2A} adenosine receptors have been previously blocked with DPCPX (10 nM) and ZM 241385 (10 nM). By itself, ZM (10 nM), applied in the presence of DPCPX (10 nM), was virtually devoid of effect on the slope of EPSPs (per cent change of the field EPSP slope: $0.2 \pm 2.5\%$, $n=3$), which confirms previous observations (Cunha *et al.*, 1997). The simultaneous perfusion of ZM 241385 (10 nM) and DPCPX (10 nM) increased EPSP slope by $25 \pm 1.2\%$ ($n=3$), an increase that was not significantly different ($P>0.05$) from that obtained ($21 \pm 4.2\%$, $n=20$) with DPCPX (10 nM) alone.

In another set of experiments we applied the A_{2A} receptor antagonist only after the full excitatory action of CGRP (30 nM) in the presence of the DPCPX (10 nM) had been observed. Under these conditions, CGRP (30 nM) increased the field EPSP slope by $18 \pm 5.4\%$ ($n=4$, $P<0.05$) and ZM 241385 (10 nM) was unable to reverse this excitatory effect of the neuropeptide. (Figure 3B).

To further evaluate how A_{2A} receptor activation by endogenous adenosine was needed for the enhancement of synaptic transmission caused by CGRP upon A_1 receptor blockade, experiments were designed to test the influence of adenosine deaminase, an enzyme that inactivates extracellular adenosine into inosine, in that action of CGRP. Adenosine deaminase (2 U ml^{-1}) was applied to hippocampal slices in the presence of DPCPX (10 nM) and, as expected from the lack of effect of the A_{2A} antagonist on field EPSPs, the enzyme was also virtually devoid of effect (per cent change of field EPSP slope: $-2.5 \pm 2.3\%$, $n=4$, after 20 min application) on field EPSP slope. In these slices, the subsequent application of CGRP (30 nM) did not appreciable affect the field EPSPs (per cent change of the slope: $1.3\% \pm 2.4$, $n=4$). As expected, in parallel slices from

the same hippocampus, GCRP (30 nM) applied in the presence of DPCPX (10 nM) but in the absence of adenosine deaminase, increased the slope of the EPSPs by 15±2.3% ($n=4$, $P<0.05$)

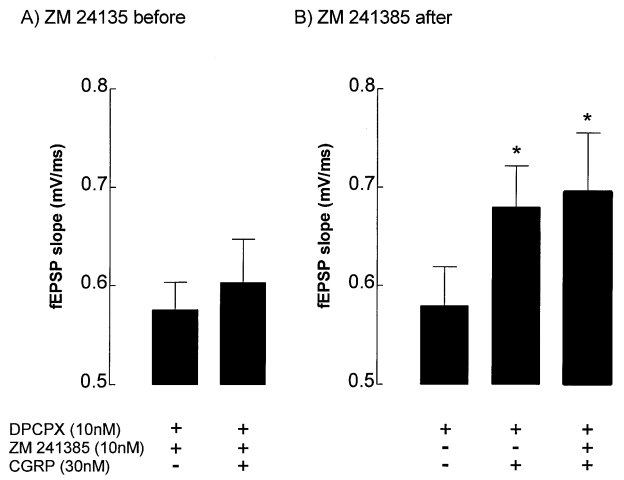

Figure 3 Influence of the blockade of A_{2A} adenosine receptors with the selective antagonist, ZM 241385 (10 nM), upon the enhancement caused by calcitonin gene related peptide (CGRP, 30 nM) on the slope of field excitatory postsynaptic potentials (fEPSPs) recorded from the CA1 area of rat hippocampal slices. (A) Represents the results obtained in the experiments ($n=3$) in which ZM241385 was applied before CGRP and (B) represents the results obtained in the experiments ($n=4$) in which ZM241385 was applied after the full effect of CGRP. The presence (+) or absence (−) of each drug is indicated below each column. In each panel the sequence of drug application corresponds to the sequence of columns. The field EPSP slope in the absence of any drugs was 0.45±0.02 mV ms^{-1} in (A) and 0.48±0.11 mV ms^{-1} in (B). *$P<0.05$ (one-way repeated measures ANOVA followed by the Newman-Keuls test) as compared with the field EPSP slope recorded in the same slices before application of CGRP (first column in each panel). Note that ZM241385 prevented (A), but did not reverse (B), the excitatory effect of CGRP.

Influence of A_{2A} receptor activation upon the effect of CGRP on hippocampal synaptic transmission

The results obtained with the simultaneous blockade of A_1 and A_{2A} receptors suggested that the induction of the excitatory effect of CGRP in the presence of DPCPX requires tonic activation of A_{2A} receptors by endogenous adenosine. To further evaluate how A_{2A} receptor activation interferes with the action of CGRP on synaptic transmission in the hippocampus, we tested the action of this neuropeptide in slices where the A_{2A} adenosine receptors have been activated by the A_{2A} agonist, CGS 21680. By itself, CGS 21680 (10 nM) increased the slope of field EPSPs by 8.7±1.7% ($P<0.05$) in seven out of 12 experiments. In five experiments CGS 21680 was virtually devoid of effect on the field EPSPs. This variability in the effect of the A_{2A} receptor agonist in the hippocampus has been previously reported (Sebastião & Ribeiro, 1992; Li & Henry, 1998).

As illustrated in Figure 4A, CGRP (30 nM) caused a marked and reversible increase in the slope of the field EPSPs when applied to hippocampal slices in the presence of the A_{2A} agonist, CGS 21680 (10 nM). The facilitation of synaptic transmission caused by CGRP (3–30 nM) in the presence of CGS 21680 (10 nM) was concentration-dependent (Figure 4B), 30 nM CGRP increasing the slope of the field EPSPs by 36±8.2% ($n=5$, $P<0.05$), an effect which is significantly larger ($P<0.05$) than that observed in other experiments using the same concentration of CGRP but in the presence of 10 nM DPCPX (17±1.3%, $n=19$). Interestingly, the ability of the A_{2A} agonist to unmask the facilitatory effect of CGRP on synaptic transmission does not appear to depend on its ability to facilitate synaptic transmission, since in the hippocampal slices where CGS 21680 (10 nM) did not increase the field EPSP slope, CGRP (3–30 nM) applied in the presence of CGS 21680 did facilitate synaptic transmission.

Influence of PK-A blockade or K_{ATP} channels blockade on the effect of CGRP on synaptic transmission

To evaluate the influence of the activity of protein kinase-A (PK-A) on the effect of CGRP we tested whether HA-1004 or

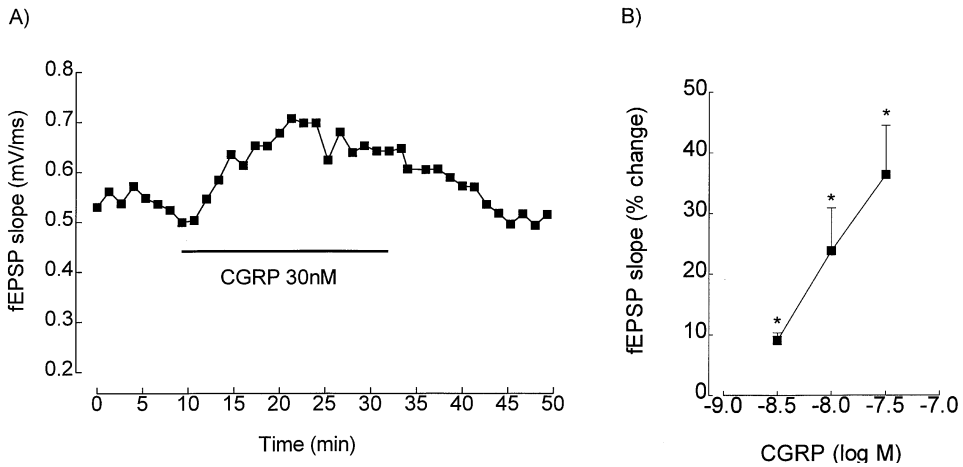

Figure 4 Enhancement caused by calcitonin gene-related peptide (CGRP) of the slope of field excitatory postsynaptic potentials (fEPSPs) recorded from the CA1 area of the rat hippocampal slices in the presence of the A_{2A} receptor agonist, CGS 21680 (10 nM). (A) Shows the time course of the effect of CGRP obtained in one slice. Each point, in the ordinates, corresponds to the slope of the average of eight consecutive field EPSPs, and in the abscissae to the start of averaging. The time of perfusion of CGRP (30 nM) is indicated by the horizontal bar and CGS 21680 (10 nM) was always present. (B) Shows the percentage enhancement of the field EPSP slope caused by different concentrations of GCRP. Each point represents the mean±s.e.m. results obtained in three to five slices obtained from different animals. The concentration-response curves were obtained in a non-cumulative way, and in each slice CGRP was applied only once, and after a stable response to CGS 21680 (10 nM) was obtained. Average field EPSP slope in control conditions (0%, presence of 10 nM CGS 21680): 0.49±0.04 mV ms^{-1} ($n=12$). *$P<0.05$ as compared with 0% (Student's t-test).

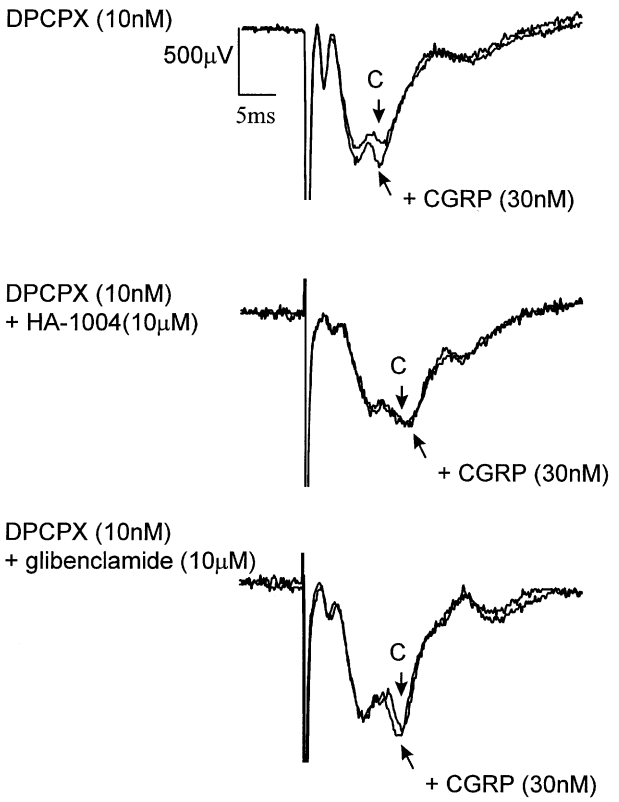

Figure 5 Blockade by the protein kinase A inhibitor, HA-1004 (10 μM), and by the inhibitor of K_{ATP} channels, glibenclamide (30 μM), of the facilitatory action of calcitonin gene related peptide (CGRP, 30 nM) on field excitatory postsynaptic potentials (EPSPs) recorded from the CA1 area of rat hippocampal slices. The recordings were obtained in the same experiment, using three hippocampal slices from the same hippocampus. Each panel shows two superimposed field EPSPs (each an average of eight consecutive responses) from the same slice obtained immediately before (control, C) and 20 min after CGRP perfusion, as indicated by the arrows. The effect of CGRP was tested first in the absence (upper panel), than in the presence of HA-1004 (middle panel) or glibenclamide (lower panel). The adenosine A_1 receptor antagonist, DPCPX (10 nM) was present throughout the experiment. In this experiment, the per cent modification of the field EPSP slope caused by HA-1004 (10 μM) and glibenclamide (30 μM) was -0.5% and -6%, respectively.

RpcAMPS, two PK-A inhibitors (Hidaka & Kobayashi, 1992; Rothermel & Parker-Bothelho, 1998), affected the ability of CGRP to enhance the slope of field EPSPs. When applied to hippocampal slices in the presence of DPCPX (10 nM), neither HA-1004 (10 μM) nor RpcAMPS (50 μM) caused appreciable changes in the slope of the field EPSPs (per cent change: $-3.5\pm3.2\%$, $n=5$, for HA-1004 and $-2.4\pm4.7\%$, $n=3$, for RpcAMPS); however, the subsequent application of CGRP (30 nM) to these slices failed to increase the slope of the field EPSPs ($1.8\pm1.7\%$, $n=5$, in the HA-1004 experiments, and $-0.76\pm3.2\%$, $n=3$, in the RpcAMPS experiments). As illustrated in Figure 5, CGRP (30 nM) increased the slope of the field EPSPs ($16\pm1.4\%$, $n=6$, $P<0.05$) when applied to parallel slices in the presence of DPCPX (10 nM) but in the absence of the PK-A inhibitors.

In Figure 5 is also illustrated the ability of the inhibitor of ATP dependent potassium channels (K_{ATP} channels), glibenclamide, to prevent the excitatory effect of CGRP on synaptic transmission. By itself, and when applied to hippocampal slices in the presence of DPCPX (10 nM), glibenclamide (30 μM) caused a decrease ($15\pm4.9\%$, $n=7$, $P<0.05$) of the slope of the field EPSPs. In six experiments using glibenclamide (30 μM) plus DPCPX (10 nM), CGRP (30 nM) failed to modify the EPSP slope in the presence of glibenclamide (per cent change: $1.7\pm0.7\%$) but increased EPSP slope ($17\pm5\%$) in the absence of the K_{ATP} channels inhibitor.

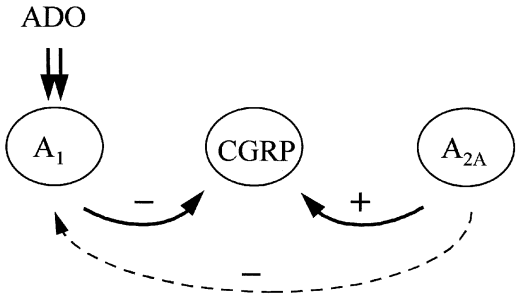

Figure 6 Schematic diagram of the model proposed for the interactions between adenosine (ADO) and CGRP in the hippocampus. Tonic activation of A_1 adenosine receptors by endogenous adenosine is indicated by the double arrow. An inhibitory interaction is indicated by (−) and a facilitatory interaction is indicated by (+).

Discussion

To observe an exciatory action of CGRP on synaptic transmission in the CA1 area of the hippocampus it was necessary to block A_1 adenosine receptors with its selective antagonist, DPCPX or to activate A_{2A} adenosine receptors with its selective agonist, CGS 21680. The enhancement of synaptic transmission caused by CGRP upon blockade of A_1 adenosine receptors requires activation of excitatory A_{2A} adenosine receptors by endogenous adenosine, since the selective A_{2A} receptor antagonist ZM 241385, or adenosine deaminase, applied before CGRP, could prevent the facilitatory effect of this neuropeptide. Interestingly, when applied after the full effect of CGRP, ZM 241385 was unable to revert the excitatory action of the neuropeptide. This precludes the remote possibility that the A_{2A} agonist was directly interfering with the CGRP receptors and suggests that tonic activation of A_{2A} adenosine receptors is required for the induction but not for the maintenance of the excitatory action of CGRP on hippocampal synaptic transmission.

The ability of DPCPX and of CGS 21680 to trigger an excitatory action of CGRP on synaptic transmission in the hippocampus does probably not result solely from their facilitatory action on synaptic transmission, since (1) the simultaneous perfusion of DPCPX and ZM 241385 also caused an increase in the field EPSP slope but, under these conditions, CGRP was unable to facilitate synaptic transmission and (2) CGS 21680 was able to trigger an excitatory action of CGRP even in the experiments where the A_{2A} agonist was unable to facilitate synaptic transmission.

A schematic diagram of the probable interplay between CGRP and A_1 or A_{2A} receptors is represented in Figure 6. It is known that the main tonus of endogenous adenosine in the hippocampus, at least in the experimental conditions used, is inhibitory (e.g. Dunwiddie & Diao, 1994; Cunha *et al.*, 1996). Thus, the absence of effect of CGRP alone and its effect in the presence of DPCPX are highly suggestive that endogenous adenosine, by activating A_1 receptors, is tonically restraining the action of CGRP. Relieve of this inhibition by DPCPX allows the initiation of the action of CGRP providing that the A_{2A} receptors are operative to be activated by endogenous adenosine. Instead of blockade of A_1 receptors, activation of

A_{2A} receptors with its agonist, CGS 21680, was also able to trigger an excitatory action of CGRP on synaptic transmission. It is known that A_{2A} adenosine receptor activation by CGS 21680 inhibits A_1 receptor functioning in the hippocampus (Cunha *et al*., 1994). Thus, the A_1 adenosine receptors are less operative in the presence of CGS 21680 and this may contribute to the ability of the A_{2A} agonist to trigger the facilitatory action of CGRP on synaptic transmission. However, this A_{2A}/A_1 adenosine receptor interaction is probably not the only mechanism by which A_{2A} receptor activation induces the ability of CGRP to enhance synaptic transmission, since the effect of this neuropeptide was greater in the presence of the A_{2A} agonist, CGS 21680, than in the presence of the A_1 antagonist, DPCPX.

CGRP receptors are positively coupled to adenylate cyclase in a variety of tissues (for a review see Wimalawansa, 1996), and evidence has been provided that stimulation of adenylate cyclase, increased production and accumulation of cyclic AMP, and activation of PK-A, mediates activation of K_{ATP} channels by GCRP (Wellman *et al*., 1998). The present observations that the excitatory action of CGRP on synaptic transmission is prevented by inhibitiors of PK-A, as well as by a K_{ATP} channel blocker, is also consistent with an involvement of PK-A and K_{ATP} channels in the action of CGRP in the hippocampus. A_{2A} adenosine receptors are in most cases positively coupled to adenylate cyclase (see Sebastião & Ribeiro, 1996). It is conceivable that A_{2A} receptor activation, by causing local enhancement of cyclic AMP levels, acts as a primer of the transducing system operated by CGRP in the hippocampus, which might explain the presently observed positive interaction between A_{2A} receptors activation and the action of CGRP on synaptic transmission.

The predominant inhibitory tonus by endogenous adenosine in the hippocampus (Dunwiddie & Diao, 1994), might explain why CGRP by itself is unable to affect synaptic transmission. This contrasts with what occurs at motor nerve terminals, where endogenous adenosine tonically activates A_{2A} receptors (Correia-de-Sá *et al*., 1996), and CGRP by itself facilitates neurotransmitter release (Correia-de-Sá & Ribeiro, 1994). Interestingly, as it occurs in the hippocampus (present results), blockade of A_{2A} receptors prevents the facilitatory action of CGRP at motor nerve terminals (Correia-de-Sá & Ribeiro, 1994).

In conclusion, the results presented in this paper show that the ability of CGRP to facilitate synaptic transmission in the CA1 area of the hippocampus is under tight control by adenosine, with the A_1 receptors 'braking' the action of CGRP and the A_{2A} receptors 'triggering' this action. The need of A_{2A} receptor activation by endogenous adenosine to reveal the excitatory action of CGRP on synaptic transmission in the hippocampus, taken together with the observation that the induction of the excitatory action of CGRP on synaptic transmission caused by CGS 21680 was more evident and consistent than the enhancement of synaptic transmission caused by the A_{2A} receptor agonist itself, suggests that a main role A_{2A} adenosine receptors in the hippocampus is to modulate the action of other neuromodulators and, by this process, to contribute for a sophisticated 'fine tune' of hippocampal functioning.

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