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# ADENOSINE–HOMOCYSTEINE INTERACTIONS IN CONTROLLING CYCLIC AMP IN RAT BRAIN CORTICAL SLICES

MICHAEL E. NEWMAN

Department of Research, Jerusalem Mental Health Center, Ezrath Nashim Hospital, P.O.B. 140, Jerusalem, Israel

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**Abstract**—Preincubation of rat brain cortical slices with homocysteine resulted in a reduction of the cyclic AMP response to subsequently added noradrenaline or isoproterenol. There was no effect of homocysteine on the adenosine response, nor of added *S*-adenosyl homocysteine on any of the responses studied. The results are interpreted in terms of binding of endogenous adenosine by the enzyme *S*-adenosylhomocysteinase, and provide further evidence for the controlling role of adenosine on cerebral cyclic AMP production.

Adenosine has been shown to have a variety of biochemical effects in cerebral and other tissues (for review see [1, 2]). Prominent among these and contributing especially to its role as a neuromodulator in the central nervous system are its effects on levels of cyclic AMP, which have been shown to be mediated by receptors either positively or negatively coupled to adenylate cyclase and named $A_1$ and $A_2$ [3] or $R_i$ and $R_a$ [4]. The characteristics of these receptors have been determined by binding experiments using various adenosine analogs chemically modified so as to increase their affinities for one or another of the specific receptor sites [5–8]. However, binding experiments using adenosine itself [9–11] show that a greater number of sites than those labelled by the modified adenosine analogs exist. It has been proposed [12] that a substantial proportion of these binding sites can be accounted for by the "adenine analog binding protein", originally found in mouse liver [13] and subsequently identified as the enzyme *S*-adenosyl homocysteinase [14], which catalyses the reversible reaction.

Adenosine + Homocysteine = *S*-adenosylhomocysteine (SAH)

This protein is capable of sequestering large amounts of added adenosine [15], and may be identical with the reported binding site for exogenous *S*-adenosylhomocysteine recently found in both brain [16] and liver [17], although it has also been suggested [18] that it is an intracellular protein only. I.p. administration of homocysteine to mice [19] has been shown to decrease cerebral adenosine levels and raise SAH levels, indicating that the condensation reaction resulting in SAH formation also occurs *in vivo*.

If a proportion of the high concentration of free adenosine in the brain [12] is indeed sequestered to form SAH, it could be expected that modification of the equilibrium catalysed by *S*-adenosylhomocysteinase would lead to changes in the ability of adenosine to increase cyclic AMP levels in brain by activation of $A_2$ receptors. In mouse lymphocytes [20], preincubation with a combination of adenosine and homocysteine was shown to markedly enhance the cyclic AMP responses to subsequently added prostaglandin $E_1$, adenosine, 2-chloroadenosine, isoproterenol and cholera toxin. This effect was shown to be related to increased intracellular levels of SAH, and to be due to both amplification of the activity of adenylate cyclase and inhibition of cyclic AMP phosphodiesterase. In a rat hippocampal slice preparation [21], SAH synthesis was shown to be only a minor pathway of adenosine metabolism, although in guinea-pig cortical slices [22], addition of L-homocysteine thiolactone significantly diminished the output of adenosine due to electrical stimulation, indicative of sequestration to form SAH. In mice, homocysteine-induced seizures have been shown to be accompanied by a rise in cyclic AMP which could be inhibited by propranolol pretreatment [23]. It therefore seemed worthwhile to investigate the effects of homocysteine and SAH on the accumulation of cyclic AMP in brain slices induced by a variety of agents. Since several of these responses are dependent on the presence of endogenous adenosine [24, 25], the experiments were intended to provide further information on the role of homocysteine in modifying free adenosine concentrations in brain.

## MATERIALS AND METHODS

Experiments were performed with male rats of the Sabra strain, 150–200 g, housed with a reversed lighting cycle and allowed *ad libitum* access to food and water. Cerebral cortices were sliced using a McIlwain tissue chopper set at 0.35 mm and preincubated in Krebs–Ringer's bicarbonate buffer containing 10 mM glucose and 1.29 mM $CaCl_2$, gassed with 95% $O_2$: 5% $CO_2$ for 30 min at 37°. The slices were then collected on a Buchner funnel and distributed among vials containing 5 ml Krebs–Ringer's with additions for 20 min. Where a preincubation period with homocysteine or *S*-adenosylhomocy-

Table 1. Effect of simultaneous addition of homocysteine on accumulations of cyclic AMP in rat cerebral cortical slices

| Agent | Cyclic AMP (pmole/mg protein) No homocysteine | +0.1 mM homocysteine |
|---|---|---|
| None | 14.1 ± 3.8 | 15.4 ± 4.1 |
| 0.1 mM noradrenaline | 57.7 ± 17.5 | 43.7 ± 13.6 |
| 0.1 mM 2-Cl-adenosine | 51.2 ± 19.6 | 42.0 ± 7.8 |
| 0.1 mM noradrenaline + 0.1 mM 2-Cl-adenosine | 106.9 ± 14.7 | 85.5 ± 22.6 |
| 0.1 mM histamine | 24.5 ± 5.8 | 20.8 ± 5.5 |

Slices were preincubated for 30 min and incubated for 20 min as described in Methods. Results are mean ± S.E.M. of 4 observations in each case.

steine was employed, the slices had 20 min with these agents before addition of other agents and a further 20 min incubation. At the end of this period the slices were transferred to test tubes, centrifuged, the supernatants decanted, and the pellets homogenized in 2 ml 95% ethanol. Aliquots of the supernatants were evaporated to dryness under $N_2$ and cyclic AMP determined by a protein binding method based on that of Brown *et al.* [26].

Homocysteine thiolactone, noradrenaline, adenosine, 2-chloroadenosine and adenosine deaminase (type III, from calf intestinal mucosa) were all obtained from Sigma Chemical Co., St. Louis, MO, U.S.A. RO-20-1724 was a gift of the Arpad Plesch Research Foundation, Vaduz, Lichtenstein.

## RESULTS

Simultaneous addition of 0.1 mM homocysteine had no significant effect on either basal levels of cyclic AMP or the degrees of stimulation produced by noradrenaline, 2-chloroadenosine, a combination of these agents, or histamine (Table 1), although comparison of mean values shows falls of up to 24% with homocysteine. The degree of stimulation produced by histamine was low, in keeping with other results on rat as opposed to guinea-pig slices [27]. When the slices were preincubated for 20 min with 0.1 mM homocysteine before addition of the stimulating agents, however, the rise in cyclic AMP levels produced by noradrenaline was significantly reduced, although there were no changes in basal levels or in the rise produced by 2-chloroadenosine (Table 2). The 2-chloroadenosine response was also unaffected by a higher concentration of homocysteine, 0.2 mM. Preincubation with the phosphodiesterase inhibitor RO-20-1724, on the other hand, resulted in a potentiation of the noradrenaline effect. Preincubation of the slices with adenosine deaminase had no effect on basal but reduced both noradrenaline-stimulated and 2-chloroadenosine-stimulated cyclic AMP levels. Preincubation with a combination of homocysteine and adenosine deaminase produced a slight reduction in basal levels and a significant reduction in the response to noradrenaline, but did not reduce the 2-chloroadenosine response beyond the level obtained with deaminase preincubation alone (Table 2). Preincubation with 0.1 mM SAH had no effect on any of the activities studied (Table 2).

The effects of homocysteine preincubation on cyclic AMP accumulations produced by isoproterenol and adenosine were similar to those on accumulations induced by noradrenaline and 2-chloroadenosine, respectively (Table 3). As with responses to adenosine and 2-chloroadenosine, homocysteine

Table 2. Effects of preincubation with homocysteine, adenosine deaminase or SAH on accumulation of cyclic AMP in rat cerebral cortical slices

| Addition to preincubation | Cyclic AMP (pmole/mg protein) on incubation with No addition (basal) | 0.1 mM noradrenaline | 0.1 mM 2-Cl-adenosine |
|---|---|---|---|
| None | 13.5 ± 2.2 (13) | 32.4 ± 3.3 (14) | 40.9 ± 3.4 (10) |
| 0.4 mM RO-20-1724 | 10.7 ± 1.4 (2) | 68.5 ± 17.7 (4)* | |
| 0.1 mM homocysteine | 14.0 ± 2.5 (12) | 22.4 ± 3.1 (14)* | 35.4 ± 5.3 (9) |
| 0.2 mM homocysteine | | | 40.9 ± 9.9 (3) |
| 2 U/ml adenosine deaminase | 12.7 ± 3.8 (4) | 22.2 ± 4.4 (4) | 27.3 ± 3.3 (4)* |
| Homocysteine + adenosine deaminase | 9.8 ± 2.5 (4) | 13.3 ± 3.8 (4)* | 29.1 ± 3.0 (3) |
| 0.1 mM SAH | 12.7 ± 3.1 (4) | 30.0 ± 2.6 (6) | 37.0 ± 5.7 (3) |

Slices were preincubated for 30 min before distribution into incubation vials, preincubated for 20 min and incubated for a further 20 min as indicated above. Results are mean + S.E.M. of the number of observations in parentheses.

* Significantly different from no addition to preincubation by Student's *t*-test ($P < 0.05$).

Table 3. Effects of preincubation with homocysteine on accumulation of cyclic AMP in rat cerebral cortical slices

| Agent | No homocysteine | +0.1 mM homocysteine |
|---|---|---|
| None | 9.2 ± 1.5 (2) | 6.0 ± 0.7 (2) |
| 0.1 mM isoproterenol | 17.0 ± 1.3 (2) | 8.5 ± 1.3 (2) |
| 0.1 mM adenosine | 29.8 ± 2.9 (2) | 29.8 ± 0.3 (2) |
| 0.1 mM noradrenaline + 0.1 mM 2-Cl-adenosine | 92.5 ± 11.4 (4) | 125.0 ± 12.6 (4) |

Slices were preincubated and incubated as described in the legend to Table 2. Results are mean ± S.E.M. or range of the number of observations in parentheses.

preincubation had no significant effect on the response to a combination of noradrenaline and 2-chloroadenosine (Table 3).

## DISCUSSION

Homocysteine thiolactone in the present experiments reduced accumulation of cyclic AMP in response to noradrenaline and isoproterenol. This effect can be explained by sequestration of the endogenous adenosine present in the slices to form SAH, thus reducing the amount of free adenosine available to contribute to the "adenosine-dependent" component of the adrenergic response [24, 25]. This component is greater in guinea pig than in rat brain slices, as shown also by the failure of adenosine deaminase in the present experiments to reduce basal cyclic AMP levels as opposed to similar experiments in guinea pig brain slices [28], and also applies to a greater extent to the $\alpha$-component of the adrenergic response than to the $\beta$-component. However, the reduction by homocysteine of the response to isoproterenol, a pure $\beta$-agonist, in the present experiments, suggests that its action is also partially dependent on the presence of adenosine, and a similar effect was found with the $\beta$-agonist 2-fluoronorepinephrine by Daly *et al.* [25], using adenosine deaminase. The almost additive inhibitions of the noradrenaline response produced by homocysteine and adenosine deaminase in the present work would seem to indicate that the two agents act on different pools of adenosine. The need for preincubation with homocysteine in order to be able to observe inhibition would indicate that homocysteine preferentially sequesters intracellular adenosine. This would be in keeping with the proposed intracellular site for *S*-adenosylhomocysteinase [18], and would argue that the site at which adenosine modifies cyclic AMP responses to other hormones or neurohumoral agents is not the externally-facing membrane receptor site but another site, perhaps the guanyl nucleotide binding protein or coupling factor of adenylate cyclase [29]. The lack of an effect of homocysteine on the responses to externally added adenosine or 2-chloroadenosine in the present experiments cannot be explained by the amount of homocysteine added being insufficient to sequester all the adenosine responsible for the cyclic AMP effect, since 0.2 mM homocysteine produced similar results. A more likely explanation is therefore the location of the homocysteine effect. According to this model, externally added adenosine acts extracellularly and is unaffected by the *S*-adenosylhomocysteinase equilibrium.

The lack of effect of added *S*-adenosylhomocysteine on the cyclic AMP responses indicates that the mechanism present in mouse lymphocytes [20], whereby accumulation of SAH results in potentiation of a variety of hormonal effects, is not present in brain slices. The effect has indeed been suggested to occur largely via inhibition of cyclic AMP phosphodiesterase, since the SAH analog 5'-deoxy-5'-*S*-isobutylthioadenosine had similar effects to SAH on cyclic AMP accumulation in intact cells, which could be entirely attributed to its effects on the phosphodiesterase as measured in homogenates [30]. Neither homocysteine nor SAH appeared to have phosphodiesterase inhibitory activity in the present experiments, as can be seen by comparison of the data for preincubation with these agents in Table 2 with that for preincubation with RO-20-1724.

The effects of homocysteine in inducing convulsions have not yet been satisfactorily explained by any biochemical mechanism. The accompanying rise in cyclic AMP levels was shown to be prevented by propranolol, indicating stimulation of $\beta$-adrenergic receptor-mediated activity [23]. However the present results provide no evidence for a similar mechanism operating in brain slices. The convulsant effects of homocysteine may however be related to activity of adenosine at $A_1$ receptors, which mediate its anticonvulsant and other behavioural depressant actions [31, 32]. Sequestration of endogenous adenosine would decrease the concentration of free adenosine available for interaction with these receptors. A similar mechanism has been proposed to account for the sleep-inducing effects of *S*-adenosylhomocysteine [33, 34] by the increase in free adenosine concentrations produced by the effect of this compound on the *S*-adenosylhomocysteinase equilibrium [12]. The compensatory increase in adenosine $A_1$ receptor number produced by chronic electroconvulsive therapy [35] indicates that the anticonvulsant effects of adenosine mediated by these receptors are indeed subject to physiological and pharmacological modification.

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