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Short communication

Role of endogenous adenosine in the expression of opiate withdrawal in rats

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Abstract

Samples of extracellular fluid from striatum and nucleus accumbens of anaesthetised rats undergoing opiate withdrawal were collected using microdialysis and then analysed for adenosine and its metabolites using high performance liquid chromatography (HPLC) and ultraviolet (UV) detection. Although the amount of adenosine present in the dialysate from either brain region was below the limit of detection by 90 min after probe placement, the metabolites could still be detected. Samples of dialysates collected from the nucleus accumbens contained significantly higher concentrations of hypoxanthine and inosine following naloxone challenge. The data are compatible with the hypothesis that endogenous adenosine might be involved in the expression of the opiate abstinence syndrome.

Keywords: Adenosine, endogenous; Opiate withdrawal

1. Introduction

There is evidence to support a role for adenosine during opiate withdrawal based on the observation that non-selective adenosine receptor agonists and inhibitors of adenosine uptake depress the naloxone-precipitated opiate withdrawal syndrome in rats (Kaplan and Sears, 1996; Salem and Hope, 1997) whilst adenosine receptor antagonists exacerbate the opiate withdrawal syndrome (Kaplan and Sears, 1996; Salem and Hope, 1997). These results suggest some kind of adenosine–morphine interaction during withdrawal and that endogenous adenosine may have a role on the expression of withdrawal behaviour. It is possible that endogenous adenosine is present and acts at its receptors to produce ongoing inhibitory 'tone' which, when blocked, results in an enhancement of withdrawal behaviour.

One possible source of extracellular adenosine in the brain during withdrawal is cyclic adenosine 3′,5′-monophosphate (cAMP). Chronic morphine treatment has been shown to result in an up-regulation of the adenylate cyclase/cAMP system in certain brain regions (Nestler, 1992, 1993). In a further study, this group found parallels in the time course of adenylate cyclase, protein kinase and behavioural signs of withdrawal, and suggested that these changes were associated with opiate-abstinence (Rasmussen et al., 1990). Since it has been shown that extracellular adenosine can arise from cAMP released from the cell and it is converted to adenosine by ectophosphodiesterase and ecto-5′-nucleotidase (Rosenberg and Dichter, 1989; Bonci and Williams, 1996), it is possible that during withdrawal cAMP may be released from brain regions where levels are elevated and may be involved in maintaining the hypothesised inhibitory 'tone', presumably following its conversion to adenosine.

In this study, samples of extracellular fluid from brains of anaesthetised rats undergoing opiate withdrawal were collected using microdialysis and concentrations of adenosine and its two metabolites inosine and hypoxanthine analysed using high performance liquid chromatography (HPLC) and ultraviolet (UV) detection. It was decided to investigate adenosine release in the nucleus accumbens, an area which shows elevated adenylate cyclase activity during opiate withdrawal (Terwilliger et al., 1991). Studies were also carried out in the rat striatum in order to provide

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a comparison, the presence of adenosine receptors having been demonstrated in this region (Cornfield et al., 1992) and extracellular adenosine levels have been monitored using microdialysis probes (Ballarin et al., 1987, 1991).

2. Methods

2.1. Animals

Male Hooded Wistar rats weighing 280–300 g were used for these studies. Morphine base was formulated into an emulsion (saline:liquid paraffin:arlacel A, 8:6:1). Animals were injected s.c. in the scruff of the neck with a total of 250 mg/kg morphine in a volume of 10 ml/kg. Half the dose was administered on the morning of the first day and the remainder on the morning of the second day. Opiate-naive rats were injected with a comparable volume of non-opioid containing (placebo or blank) emulsion. The experimental protocol described in this study was approved by the Standing Committee on Ethics in Animal Experimentation (Monash University, Australia).

2.2. Microdialysis

At the end of the 48-h treatment period, rats were anaesthetised with 60 mg/kg i.p. pentobarbitone. The trachea was cannulated to allow artificial respiration and the jugular vein for administration of drugs. Animals were placed on heat pad to maintain body temperature at 37°C and the head was fixed in a stereotaxic frame (Kopf instruments). The skull was exposed, bregma located and a microdialysis probe (CMA 12; Carnegie Medicine, Sweden) was implanted into either the nucleus accumbens (from bregma: 2.7 mm rostral, 1.5 mm lateral (right), at a depth of 7.6 mm from dura) or striatum (from bregma: 0.5 mm rostral, 2.9 mm lateral (right), at a depth of 7.0 mm from dura). The probe was perfused with Ringer solution (140.0 mM NaCl, 3.0 mM KCl, 2.2 mM $CaCl_2$, pH 6.0) at a rate of 2.0 μl/min (CMA 100 microinjection pump). Following the 2-h equilibration period, two 10-min samples of dialysate were collected. These two samples of dialysate were used as controls. At the end of the second 10-min sample collection, an i.v. bolus injection of 3 mg/kg of naloxone was given via the jugular cannula and a further two 10-min samples were collected. A total of 20 μl aliquots were injected onto the HPLC column for separation and analysis on the same day. The concentrations of adenosine, hypoxanthine and inosine were then compared to those found in the controls. Differences in the concentrations of adenosine and its metabolites between the two brain regions and before and after naloxone injection were evaluated using Student's two-tailed *t*-test using $P < 0.05$ as an indicator of significance. At the end of each experiment, a red dye was injected into the region were the probe was placed using a 23-gauge needle with the bevel removed. Animals were then killed by an overdose of pentobarbitone administered via the jugular cannula. Brains were removed and then stored (−4°C) until required. To confirm probe placement, frozen brains were cut into 0.5–1.0 mm slices starting from the 2-cm rostral to the dye spot and continuing until the dye was located inside the brain and then examined under a light microscope. Data obtained from rats in which the probe was not correctly positioned were discarded.

In order to determine in vitro recovery of adenosine and its metabolites, the microdialysis probe was placed into Ringer solution containing adenosine, hypoxanthine and inosine in concentrations ranging between 0.3 and 10 μM and maintained at 37°C. Probes were perfused with the Ringer solution for 30 min at a flow rate of 2 μl/min and the dialysate collected over 10-min intervals.

2.3. HPLC equipment and the mobile phase

Chromatographic equipment consisted of BAS 200A Liquid Chromatograph, a variable wavelength UV detector (254 nm) and Phenomenex Ultracarb 5 ODS column (150 × 4.6 mm; 5 μm). The mobile phase consisted of 0.01 mol/l sodium dihydrogen orthophosphate buffer containing 8% methanol (pH 4.5) and flow rate was 0.5 ml/min.

2.4. Drugs

Morphine hydrochloride (Macfarlane Smith), light liquid paraffin oil (BDH), naloxone, arlacel A, inosine, hypoxanthine (Sigma), pentobarbitone sodium (Boehringer Ingelheim), adenosine (Research Biochemicals, USA).

3. Results

3.1. HPLC analysis of standard adenosine and its metabolites

Adenosine, hypoxanthine and inosine were eluted after 3, 4.5 and 10 min, respectively. Standard curves for adenosine, hypoxanthine and inosine were linear over the concentration range of 300–3000 nM with correlation coefficients of 0.989, 0.992 and 0.990, respectively. Within- and between-run coefficients of variation were 2.4% and 3.6%, respectively. The limit of detection for adenosine, hypoxanthine and inosine was found to be 93, 91 and 46 nM, respectively.

3.2. In vitro recovery of adenosine and its metabolites

The recovery rates from Ringer solution (37°C) for adenosine, hypoxanthine and inosine were 13.86 ± 0.87%,

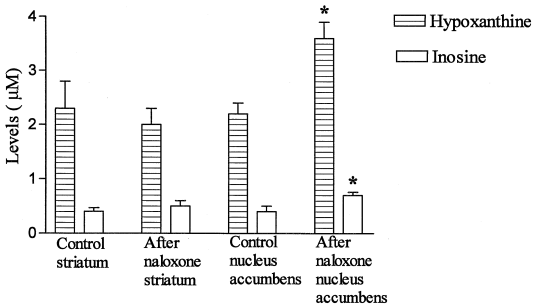

Fig. 1. Concentrations (μM; mean ± S.E.M., $n = 4$) of hypoxanthine and inosine in dialysates from striatum and nucleus accumbens of morphine-dependent rats before and after naloxone injection. Asterisks indicate that there was a statistically significant difference ($P < 0.05$).

12.45 ± 0.64% and 11.34 ± 0.32% (mean ± S.E.M., $n = 4$), respectively.

3.3. Effects of naloxone challenge

In both brain regions, the first 30 min sample of dialysate collected following probe placement contained very high concentrations of adenosine (3.3 ± 0.23 μM), hypoxanthine (9.5 ± 0.3 μM) and inosine (4.0 ± 0.1 μM). Concentrations of these compounds declined rapidly over the next 60 min and by 90 min, concentrations of adenosine fell below the limit of detection, whilst the metabolites were still present in detectable amounts. There were no statistically significant differences ($P > 0.05$, $n = 4$) in the concentration of any of these compounds between the two brain regions.

Following naloxone administration, the samples of dialysates collected from the probe implanted in the nucleus accumbens contained a significantly higher ($P < 0.05$, $n = 4$) concentration of hypoxanthine and inosine compared to the samples collected before naloxone injection (see Fig. 1). Administration of naloxone had no effect on the concentration of hypoxanthine or inosine in the dialysates collected from the striatum.

4. Discussion

In agreement with other workers (Ballarin et al., 1987; Hagberg et al., 1987), the level of adenosine in the dialysate was high immediately following placement of the probe. It has been suggested that the initially high levels were due to the trauma associated with implanting the probe and that basal levels of adenosine are reached 75–100 min after probe placement. The amount of adenosine present in the dialysate from either brain region was below the limit of detection by 90 min, the metabolites, however, could still be detected. Levels of hypoxanthine and inosine in dialysate from nucleus accumbens of morphine-dependent rats were significantly higher following i.v. naloxone than levels in dialysate from this region when no naloxone was given. This increased metabolite level following naloxone challenge was not seen in dialysates from nucleus accumbens in control rats and was also not seen in striatal dialysates from either morphine-dependent or control rats.

According to Ballarin et al. (1991), adenosine is metabolised extracellularly prior to its appearance in the dialysate so it seems reasonable to assume that the presence of the two metabolites in dialysate samples is indicative of the presence of adenosine. Whilst it was not possible to determine whether adenosine levels were altered in the dialysates from these animals, as has been explained above, it is interesting to note that the increase in the levels of these two metabolites was only observed in the nucleus accumbens, a brain region which is known to be involved in opiate dependence and withdrawal and which is also reported to have high levels of adenylate cyclase and cAMP-dependent protein kinase during chronic morphine administration (Terwilliger et al., 1991). It is thus possible that the increased basal level of (presumably) adenosine was due to efflux during withdrawal of the excess cAMP from this region. A more precise elucidation of the mechanism involved may be obtained in further experiments using probenecid, an agent which inhibit cAMP efflux or to increase cAMP metabolism by using ecto-5′-nucleotidase (Bonci and Williams, 1996).

Although in the present study administration of naloxone had no effect on the concentration of hypoxanthine or inosine in the dialysates collected from the striatum of opiate-dependent animals, it has been shown that an increased cAMP production occurs in rat striatum during opiate withdrawal (De Vries et al., 1993; Tjon et al., 1994). This discrepancy can be explained at least in part by the fact that whilst in our experiment the adenosine metabolites were measured in brains of anaesthetised rats within a subregion of the striatum (due to anatomical specificity of the microdialysis probe placement), these workers were investigating dopamine D-1 receptor stimulated cAMP production in rat striatal slices obtained from rats treated chronically with morphine. Possibly there are differences in cAMP production which occur in striatal slices as opposed in anaesthetised animals and up-regulation of adenylate cyclase activity might not be uniform within the entire striatum.

In summary, despite the inability to clearly define the role of endogenous adenosine during the naloxone-precipitated withdrawal syndrome, data obtained from the present study are in accord with the hypothesis that endogenous adenosine could be involved in this phenomenon.

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