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Amitriptyline enhances extracellular tissue levels of adenosine in the rat hindpaw and inhibits adenosine uptake

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Abstract

Local administration of amitriptyline into the rat hindpaw produces peripheral antinociception; this is reduced by adenosine receptor antagonists and appears to involve endogenous adenosine. The present study used peripheral microdialysis: (a) to determine whether amitriptyline could enhance extracellular tissue levels of endogenous adenosine in the rat hindpaw and (b) to examine mechanisms by which such an increase could occur. Local injection of amitriptyline into the plantar hindpaw, at doses that produce peripheral antinociception (100–300 nmol), produced an increase in local extracellular levels of adenosine. When injected in combination with formalin, which also enhances such levels of adenosine, an additive increase was observed. This adenosine originated partly as nucleotide, as inhibition of ecto-5′-nucleotidase reduced the amount of adenosine detected in the probe following administration of amitriptyline. When administered in combination with exogenous adenosine, amitriptyline augmented recovery of adenosine in the probe. Pretreatment of rats with capsaicin augmented the ability of amitriptyline to increase adenosine levels detected in the dialysis probe; it also enhanced tissue recovery of exogenously administered adenosine. In uptake studies using cultured rat C6 glioma cells, amitriptyline inhibited adenosine uptake by an adenosine transporter (IC_{50} 0.37±0.12 mM). In enzyme assays, amitriptyline had no effect on adenosine kinase or adenosine deaminase activity. These results demonstrate that amitriptyline: (a) enhances extracellular tissue levels of adenosine in the rat hindpaw following local administration in vivo and (b) inhibits adenosine uptake but has no effect on metabolism in vitro. Therefore, increased extracellular adenosine levels in vivo appear to result partially from extracellular conversion of nucleotide and partially from inhibition of uptake.

Keywords: Amitriptyline; Adenosine; Microdialysis; Adenosine uptake; Adenosine kinase; Adenosine deaminase

1. Introduction

Amitriptyline is used widely to treat chronic pain in humans (Bryson and Wilde, 1996; McQuay et al., 1996). Amitriptyline is a complex drug, exerting a range of pharmacological effects; these include inhibition of noradrenaline, 5-hydroxytryptamine and adenosine uptake, binding to opioid receptors, blockade of α-adrenergic, histaminic, cholinergic and *N*-methyl-D-aspartate receptors, and blockade of ion channels; many of these actions are implicated in antinociceptive properties when amitriptyline is given systemically or spinally (reviewed Sindrup, 1997; Eschalier et al., 1999; Sawynok et al., 2001). Amitriptyline also produces peripheral antinociception when administered locally to the rat hindpaw. This has been demonstrated at doses of 100–300 nmol in the rat formalin test (Sawynok et al., 1999) and in two different models of neuropathic pain (Esser and Sawynok, 2000; Ulugol et al., 2002). Local anesthetic actions, in addition to antinociception, occur at higher doses (>1000 nmol; Gerner et al., 2001; Khan et al., 2002). Antinociception at lower doses of amitriptyline is inhibited by methylxanthine adenosine receptor antagonists (Sawynok et al., 1999; Esser and Sawynok, 2000; Ulugol et al., 2002), suggesting an involvement of endogenous adeno-

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sine in peripheral antinociception. Antidepressants inhibit adenosine uptake in vitro (Phillis and Wu, 1982) and augment electrophysiological (Sattin et al., 1978; Stone and Taylor, 1979; Phillis, 1984) and behavioral actions of adenosine in vivo (Pareek et al., 1994). These observations suggest that block of adenosine uptake might be a plausible explanation for methylxanthine sensitivity of antidepressant actions when administered peripherally.

In the present study, we used a peripheral microdialysis model to examine the effect of amitriptyline on extracellular tissue levels of adenosine in the rat hindpaw in vivo and then addressed a number of potential mechanisms by which an increase in extracellular tissue levels of adenosine could occur. Amitriptyline was given both alone and in combination with formalin; the latter condition was of interest because formalin itself provokes local release of adenosine (Liu et al., 2000a) and amitriptyline suppresses formalin-evoked behaviors (Sawynok et al., 1999). The metabolic origin of adenosine was determined using α,β-methylene–adenosine diphosphate (α,β-MeADP) to inhibit ecto-5′-nucleotidase (Zimmermann, 2000), while its potential anatomical origin from sensory afferents was examined using capsaicin (C-fibre neurotoxin) (Liu et al., 2001). In vitro studies determined effects of amitriptyline on adenosine uptake by a transporter system in cultured rat C6 glioma cells, which contain the rat equilibrative nucleoside transporter rENT2 (Sinclair et al., 2001), and on the activity of adenosine kinase and adenosine deaminase, two enzymes prominent in adenosine metabolism. A preliminary report of these microdialysis studies was published as an abstract (Liu et al., 2000b).

2. Methods

2.1. Animals

Experiments were conducted using male Sprague Dawley rats 200–300 g from Charles River, Quebec. Rats were housed under standard conditions (12/12 h light/dark cycle, at 22±2 °C). All procedures were approved by the University Committee on Laboratory Animals and adhered to guidelines issued by the Canadian Council on Animal Care.

2.2. Capsaicin pretreatment

Capsaicin (10 mg/ml in 10% ethanol, 10% Tween 80 and saline) or vehicle treatments were administered s.c. for 3 consecutive days (30, 50 and 70 mg/kg, respectively), under loose neck skin under pentobarbital anesthesia (45 mg/kg). Microdialysis was performed on day 4. This protocol substantially reduces formalin-induced c-*Fos* expression in the superficial dorsal horn, indicating nociceptive sensory transmission is largely blocked (Zhang et al., 1998), and completely suppresses release of adenosine by 1.5% formalin (Liu et al., 2001).

2.3. Microdialysis experiments

Subcutaneous microdialysis experiments were performed as previously described (Liu et al., 2000a). Rats were anesthetized with pentobarbital (45 mg/kg i.p. for induction, 10 mg/kg i.p. per 30 min for maintenance) and body temperatures were maintained throughout the procedure. The microdialysis probe (LM-5 linear probe, 5-mm active membrane length, 320-μm OD, 35 kDa wt. cut-off, Bioanalytical Systems, U.S.A.) was implanted into the subcutaneous area of the plantar surface of the rat hindpaw. The probe was perfused with standard Krebs–Henseleit solution at a rate of 2 μl/min. Following a pre-perfusion for 2 h and two baseline collections, drugs were injected in a volume of 50 μl via a 30 gauge needle approximately 1 mm lateral and parallel to the probe and samples were collected at 10 min intervals into a tube containing 10 μl 0.15 M $ZnSO_4$ at room temperature. Samples were deproteinated by the addition of 10 μl 0.15 M $Ba(OH)_2$ and adenosine levels were measured by high performance liquid chromatography following generation of an etheno derivative of adenosine. A previous study indicates that adenosine exhibits a 33% recovery rate from the microdialysis fibre in vitro (Liu et al., 2000a). In vivo recovery rates are lower due to tissue uptake and/or metabolism (see text).

Data are presented as a time course of extracellular adenosine levels detected in the microdialysis probe or as a cumulative increase determined by subtracting the mean of two baseline values from subsequent values (to 60 min). Multiple group comparisons were made using analysis of variance followed by the Student Newman Keuls test, while two groups were compared using Student's *t*-test.

2.4. Adenosine transport studies

Rat C6 glioma cells, which contain rENT2, were used as described recently (Sinclair et al., 2001) and effects of amitriptyline on adenosine uptake by rENT2 were determined. Cells were cultured in 12-well plates until confluent; they were then washed twice in buffer (mM: NaCl 118, HEPES 25, KCl 4.9, K_2HPO_4 1.4, $MgCl_2$ 1.2, $CaCl_2$ 1, and glucose 5, pH 7.4 with NaOH) and preincubated with 0.5 ml buffer, amitriptyline (10 μM–3 mM) for 10 min at 22 °C. Dipyridamole (30 μM) was used to maximally inhibit rENT2. [^{3}H]–Adenosine (2 μM) was prepared in drug solutions and 0.5 ml was added to cells for a final concentration of 1 μM [^{3}H]–adenosine. Cells were incubated with [^{3}H]–adenosine for 30 s, then solutions were aspirated and cells were washed twice with ice-cold buffer. Cellular protein was dissolved by incubating cells overnight in NaOH (1 M, 0.5 ml) at 37 °C. Separate aliquots of the dissolved cells were used for protein determinations (Bradford assay) and for liquid scintillation spectroscopy. Uptake values were determined from the radioactivity in the dissolved cells and are expressed as picomole per milligram cellular protein using the specific activity of the uptake buffer. Data were analyzed by non-linear regression using the software package GraphPad PRISM Version 4.0. Statistics were performed using ANOVA followed by Tukey's post hoc test.

2.5. Enzyme activity determinations

Adenosine kinase assays were performed as described by Sinclair et al. (2000). Briefly, rat C6 glioma cells were homogenized in ice-cold 50 mM Tris–HCl (pH 7.4) and centrifuged at

38,000 ×*g* (1 h, 4 °C). Supernatants were retained as cytosolic protein. Assay reaction mixtures (100 μl) contained 50 mM Tris–HCl (pH 7.4), 0.1% bovine serum albumin, 500 nM EHNA (or *erythro*-9-(2-hydroxy-3-nonyl)adenine hydrochloride), 50% glycerol, 1.6 mM $MgCl_2$, 50 mM 2-mercaptoethanol, 50 mM KCl, 1.2 mM ATP, 2 μM (0.25 μCi) [^{3}H]–adenosine and 2 μg cytosolic protein in the presence or absence of amitriptyline. Reactions were initiated by addition of cytosolic protein and, after incubation at 37 °C for 5 min, terminated by heating to 90 °C for 10 min. Reaction products (20 μl) were spotted on DE81 ion exchange filters, dried, and washed sequentially with 1 mM NH_4COOH, distilled deionized water and 100% ethanol (2 × 5 ml each). HCl (0.25 ml, 0.2 M) and KCl (0.25 ml, 0.8 M) were then added to the filters to elute [^{3}H]–adenine nucleotides and the tritium content was determined by scintillation spectroscopy.

Adenosine deaminase assays were modified from Padua et al. (1990). Rat C6 glioma cells were trypsinized, centrifuged and resuspended in low citrate buffer (25 mM sodium citrate, 50 mM KCl, pH 6.0, 4 °C). Cells were then homogenized and centrifuged at 12,000 ×*g* and supernatants were collected. Samples consisted of cytosolic protein and 100 μM adenosine with or without amitriptyline or 10 μM EHNA and were incubated at 37 °C for 30 min. Reactions were terminated by addition of trichloroacetic acid, then neutralized with KOH. HPLC (Waters LC Module 1 plus) was used to measure adenosine and inosine. HPLC was performed with a Waters Spherisorb S5 ODS2 4 × 240 mm analytical column, a running buffer of 10 mM $NH_4H_2PO_4$ and 14% methanol (pH 6.0), and a flow rate of 1 ml/min.

2.6. Drugs

Amitriptyline hydrochloride, formalin, adenosine, AMP, αβ-MeADP and capsaicin were purchased from Sigma. All drugs injected locally into the hindpaw were dissolved in saline. The solvent regimen for capsaicin is described in Section 2.2.

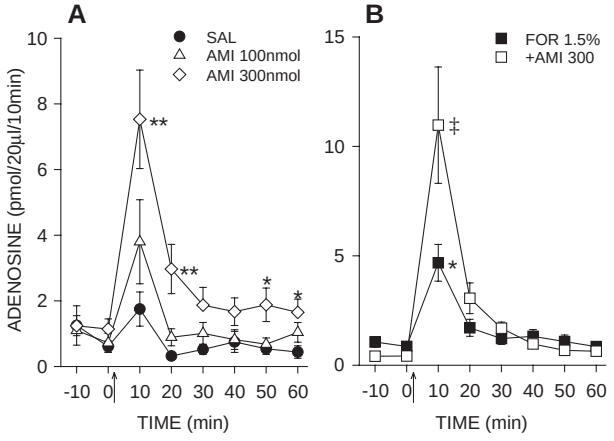

Fig. 1. Time course of the effect of amitriptyline, administered locally to the rat hindpaw, on extracellular adenosine levels determined by peripheral microdialysis. In (A), amitriptyline (AMI) or saline (SAL) was injected at time=0, while in (B) formalin (FOR) or formalin plus amitriptyline was injected. Values represent mean ± SEM (n=6–11); *P<0.05 and **P<0.01 compared to saline. ‡P<0.05 compared to formalin.

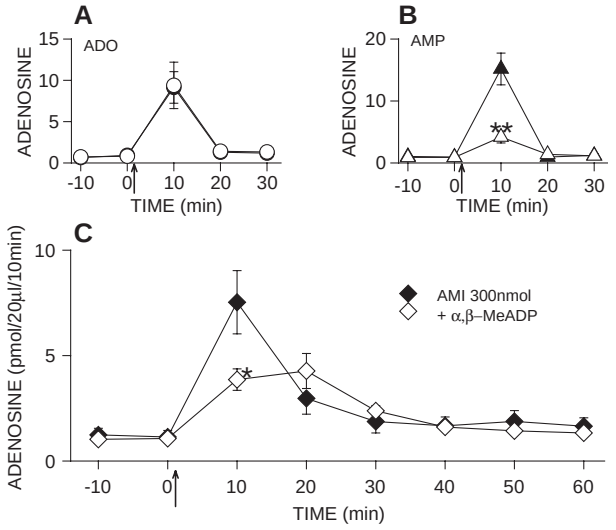

Fig. 2. Detection of adenosine in the microdialysis probe following injection of (A) adenosine (ADO) or (B) AMP (both at 5 nmol) into the rat hindpaw in the absence (solid symbols) or presence (hollow symbols) of α,β-MeADP 50 nmol to inhibit ecto-5′-nucleotidase. In (C), amitriptyline (AMI) was injected in combination with α,β-MeADP. (A and B) n=6–9 and (C) n=11–14 per group; *P<0.05 and **P<0.01 compared to the absence of α,β-MeADP.

3. Results

3.1. Effects of amitriptyline on extracellular tissue levels of adenosine

Injection of amitriptyline, 100 and 300 nmol, into the rat hindpaw produced a dose-dependent (P<0.05) increase in local tissue extracellular levels of adenosine as determined by peripheral microdialysis (Fig. 1A). This was observed most clearly within the first 10 min, but significant increases also occurred at later times at 300 nmol. Increasing the dose to 1000 nmol did not result in any further enhancement of levels (peak 7.5 ± 1.5 pmol/20 μl/10 min, n=6). Local injection of 1.5% formalin produced an increase in extracellular adenosine levels compared to saline and amitriptyline further enhanced adenosine levels when coadministered with formalin (Fig. 1B).

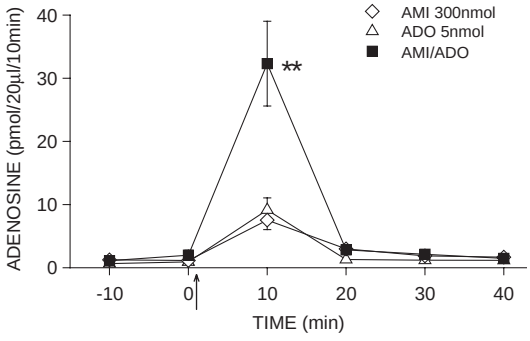

Fig. 3. Recovery of adenosine in the microdialysis probe when adenosine (ADO) and amitriptyline (AMI) are administered into the hindpaw alone or as a combination. n=6–11 per group; **P<0.01 compared to both individual groups.

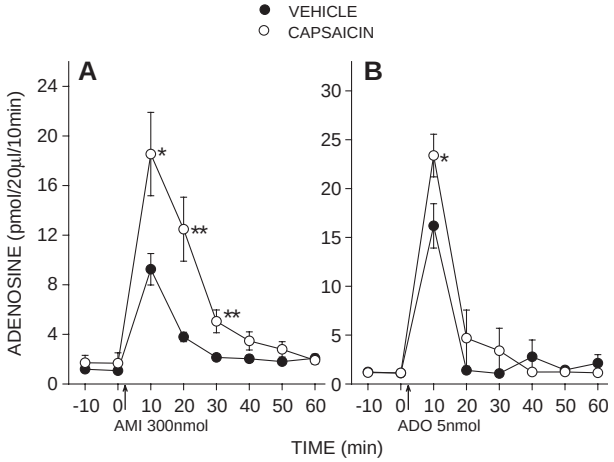

Fig. 4. Effect of pretreatment with capsaicin on the increase in adenosine following the local injection of (A) amitriptyline (AMI) or (B) adenosine (ADO). Systemic capsaicin pretreatments occurred on days 1, 2 and 3 and microdialysis was performed on day 4. $n=7-9$ per group; $^{*}P<0.05$ and $^{**}P<0.01$ compared to vehicle pretreated group.

3.2. Metabolic origin of adenosine

The metabolic origin of adenosine was examined using α,β-MeADP to inhibit ecto-5′-nucleotidase which converts AMP to adenosine. Local injection of adenosine into the hindpaw resulted in the appearance of adenosine in the microdialyis probe; coadministration of α,β-MeADP with adenosine had no effect on the appearance of adenosine in the probe (Fig. 2A). Local injection of AMP resulted in a similar appearance of adenosine in the probe indicating an efficient metabolic conversion to adenosine in the tissue; in this case, the appearance of adenosine was significantly reduced by coadministration of α,β-MeADP (Fig. 2B). When coadministered with amitriptyline, α,β-MeADP significantly reduced the peak of adenosine detected in the probe (Fig. 2C).

The administration of exogenous adenosine into the hindpaw results in <1% recovery in the microdialysis probe (Fig. 2A) and this is much lower than the 33% in vitro recovery from solutions (Liu et al., 2000a). To determine if the limited in vivo recovery reflected avid tissue uptake, we coadministered amitriptyline (inhibits adenosine uptake, see below) with adenosine to see if this would enhance recovery. There was a substantial increase in recovery of adenosine when amitriptyline was coadministered with the adenosine (Fig. 3).

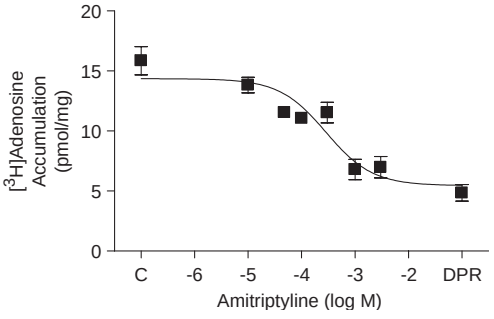

Fig. 5. Inhibitory effects of amitriptyline on adenosine uptake by cultured rat C6 glioma cells. Control (C) conditions indicate uptake in the absence of amitriptyline or dipyridamole (DPR). Values depict mean±SEM for $n=6$ independent experiments; where error bars do not appear, they are contained within the symbol. Uptake was significantly inhibited ($P<0.01$) at all concentrations above 10 μM.

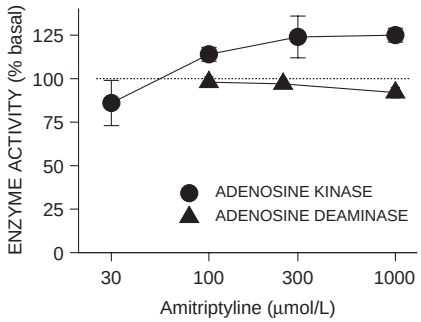

Fig. 6. Lack of effect of amitriptyline on metabolism by adenosine kinase or adenosine deaminase. Values depict mean±SEM of 3 independent experiments.

3.3. Neuronal origin of adenosine

The potential influence of C-fibres on tissue availability of adenosine was examined using a systemic pretreatment with the sensory neurotoxin capsaicin. Capsaicin pretreatment substantially augmented the amount of adenosine detected following local administration of amitriptyline (Fig. 4A). To determine whether the observed increase reflected a loss of adenosine uptake/metabolism, exogenous adenosine was injected into the hindpaw of vehicle- or capsaicin-pretreated rats; this revealed an augmented recovery of adenosine in the probe following capsaicin (Fig. 4B).

3.4. Effects of amitriptyline on adenosine uptake and metabolism in vitro

The effect of amitriptyline on adenosine uptake was determined in cultured rat C6 glioma cells containing rENT2. Amitriptyline produced a substantial inhibition of adenosine uptake and this was comparable to that observed with dipyridamole (30 μM) (Fig. 5). At all doses greater than 10 μM, this reduction was significantly different from control uptake values ($P<0.01$). The IC_{50} value for inhibition of adenosine uptake was 0.37±0.12 mM ($n=6$). In experiments using C6 cells transfected with cDNA for rENT1 (Sinclair et al., 2001), amitriptyline exhibited similar potency for inhibition of rENT1 and rENT2 (data not shown). There was no effect of amitriptyline on the activity of adenosine kinase or adenosine deaminase (Fig. 6).

4. Discussion

4.1. Amitriptyline enhances extracellular tissue availability of adenosine

The present study indicates that local administration of amitriptyline into the rat hindpaw, at doses that produce peripheral antinociception, enhances local extracellular tissue levels of adenosine. The adenosine appears to originate as nucleotide, which is subsequently converted extracellularly to adenosine via ecto-5′ nucleotidase and may further involve inhibition of adenosine uptake from the extracellular space

(see below). These increased local levels of adenosine contribute to peripheral antinociception by amitriptyline, as antinociception is inhibited by adenosine receptor antagonists, and this involves adenosine A_1 receptors based on antagonist profiles (Sawynok et al., 1999; Esser and Sawynok, 2000; Ulugol et al., 2002). [Adenosine A_{2A}, A_{2B} and A_3 receptors are unlikely to be involved, as these produce facilitatory effects on peripheral nociception (reviewed Sawynok, 1998)]. While a direct interaction of antidepressants with adenosine A_1 receptors is also a possibility in accounting for the antagonist data, there is no data in the literature to support such a mechanism. We therefore focus the following interpretations on the increase in endogenous extracellular availability of adenosine that was observed using tissue microdialysis.

The observed enhancement of adenosine levels with amitriptyline generally is shorter lasting (10–20 min) than inhibition of antinociception by adenosine receptor antagonists, which occurs over 45–60 min in the formalin test (Sawynok et al., 1999). There are two considerations. *(a) Tissue factors*. The amount of adenosine recovered in the microdialysis probe may be limited by the nature of the system used to collect it. Thus, adenosine generated extracellularly is subject to tissue uptake and/or metabolism and this limits the amount recovered by the probe. The in vivo recovery of exogenous adenosine administered to the hindpaw is <1%, even though the in vitro recovery rate for the microdialysis probe is about 33% (Liu et al., 2000a). The short duration of the recovery following the exogenous administration of substantive amounts of adenosine and of the larger amounts recovered in the presence of amitriptyline plus adenosine is a further indicator of the avid nature of the uptake (and/or metabolism). Amitriptyline, which inhibits adenosine uptake in in vitro experiments, substantially increased the in vivo recovery of adenosine, and this provides direct support for the notion that tissue uptake limits in vivo recovery. *(b) Other examples*. In other instances, the same issue of transient release and prolonged methylxanthine sensitivity of behaviors occurs. Thus, local administration of glutamate (Liu et al., 2002) or formalin (Liu et al., 2000a) to the hindpaw results in increased levels of adenosine in the microdialysis probe over 10 min, yet methylxanthine modulation of behaviors in the presence of formalin/glutamate occurs over 45 min (Aumeerally et al., 2004). Both sets of observations suggest that the dissociation in time course between probe recovery and behaviors reflects an inherent limitation in the methodology for detecting extracellular tissue adenosine levels. However, it is important to recognize that the dissociation between adenosine levels and behavior could also reflect down-stream events that obscure a more direct association from being observed.

4.2. Metabolic and cellular origin of adenosine

Extracellular adenosine can either be transported as such from the cell via ENTs or be generated outside the cell from nucleotides that have been released by exocytosis (e.g. ATP) or carrier proteins (e.g. cyclic AMP) (Latini and Pedata, 2001). While a number of enzymes metabolize nucleotides, ecto-5′-nucleotidase is the major enzyme responsible for the formation of extracellular adenosine (Zimmermann, 2000). This is inhibited by α,β-MeADP and use of this agent enables one to distinguish between adenosine and nucleotide sources for extracellular adenosine (Latini and Pedata, 2001). In the present study, AMP was efficiently converted to adenosine following local injection into the hindpaw, as coadministration of α,β-MeADP almost completely prevented its conversion to adenosine (but had no effect when adenosine was injected into the tissue, indicating the reduction was unlikely to be a non-specific effect.) When coadministered with amitriptyline, α,β-MeADP inhibited the peak amount of adenosine recovered in the probe, indicating that adenosine partially originates as nucleotide. The lack of effect at 20 min could result from diffusion of α,β-MeADP into the tissue away from the immediate vicinity of the microdialysis probe. The nucleotide released by amitriptyline may be cyclic AMP. Thus, antidepressants increase cyclic AMP formation in brain tissue (Kodama et al., 1971; Huang and Daly, 1972; Sattin et al., 1978) and this could lead to efflux of cyclic AMP from the cell via a distinct carrier (Rosenberg and Li, 1995; Van Aubel et al., 2002) with subsequent extracellular conversion to adenosine. The increase in cyclic AMP could be secondary to inhibition of the uptake of noradrenaline, 5-hydroxytryptamine or adenosine, which then activate respective G-protein coupled receptors on the cell surface to increase cyclic AMP formation inside the cell. ATP is a further possible nucleotide but, in view of reports that tricyclic antidepressants *inhibit* evoked ATP release from chromaffin cells (Izaguirre et al., 1997), this possibility is less likely.

We also determined whether the adenosine originates from sensory afferents using capsaicin, which clearly reveals a pool of adenosine can originate from sensory afferents (Liu et al., 2001). Capsaicin did not reduce the increase in adenosine availability, suggesting that the nucleotide/adenosine generated by amitriptyline originates from cellular sources other than C-fibres. These could include endothelial cells, platelets, neutrophils, or mast cells, which are known to release adenosine and/or nucleotides in peripheral tissues (Bodin and Burnstock, 2001; Sawynok, 2003). Capsaicin pretreatment actually produced an *increase* in adenosine availability following amitriptyline and in the recovery of exogenously administered adenosine. This suggests that C-fibres exert some form of limiting function on extracellular adenosine (e.g. site of uptake) and, once this is removed, an enhancement in adenosine recovery can occur. In the spinal cord, ENT binding sites are localized on capsaicin-sensitive fibres (Geiger and Nagy, 1985) and are selectively concentrated in lamina II where C-fibres terminate (Ackley et al., 2003), which is consistent with such a notion.

4.3. Effects on uptake and metabolism of adenosine

Mechanisms potentially involved in the increase in adenosine availability by amitriptyline were further examined in vitro by determining effects on adenosine uptake directly and on the activity of prominent enzymes involved in adenosine metabolism (adenosine kinase and adenosine deaminase). Amitriptyline inhibited adenosine uptake by ENT2 in cultured rat C6 glioma cells, confirming earlier studies with brain homogenates using other antidepressants (Phillis and Wu, 1982). However, the degree of inhibition with cultured cells (comparable to dipyridamole) is greater than that observed with homogenates where IC_{20}, but not IC_{50}, values were reported. While substantial inhibition of uptake requires relatively high concentrations (IC_{50} 0.37 mM), significant inhibition occurs in the micromolar range. Given that adenosine appears to be subject to avid tissue uptake in vivo, even a limited degree of inhibition of uptake could result in increased tissue recovery. Fig. 3 indicates that local administration of amitriptyline (at a dose that produces local antinociception) does lead to a marked enhancement of in vivo recovery of adenosine and this is consistent with inhibition of adenosine uptake occurring in the hindpaw under relevant experimental conditions. One must also recognize that with localized drug delivery methods, relatively high local tissue concentrations can occur initially. Thus, if the 300 nmol dose of amitriptyline were to be evenly distributed to the entire hindpaw (paw volume ~1 ml), this would lead to tissue levels of 200–300 μM and this would inhibit adenosine uptake to some extent. The lack of effect of amitriptyline on adenosine kinase and adenosine deaminase activity in vitro provides further support for an action on uptake rather than metabolism.

4.4. Further consideration of mechanisms involved in increased adenosine availability

Antidepressants in general potentially can alter extracellular availability of adenosine by further, less direct, mechanisms. Thus, antidepressants activate mechanisms that can lead to an increase in protein kinase C activity (Shimizu et al., 1993; Fukuda et al., 1994; Joshi et al., 1999), which could subsequently stimulate adenosine transport by ENT1 (Coe et al., 2002), stimulate 5′-nucleotidase inside the cell (Obata et al., 2001) or inhibit adenosine kinase (Sinclair et al., 2000). However, antidepressants also have been reported to inhibit protein kinase C activity in some tissues (Kumar et al., 1997; Vaitla et al., 1997) and effects on adenosine production via such mechanisms could be tissue and stimulus dependent.

The possibility that the observed increase in extracellular tissue adenosine levels reflects a non-specific tissue lytic effect leading to release of nucleotide from cells also needs to be considered. However, this concept is not consistent with a number of observations. Thus, if this were the case, one would expect the highest dose to produce the greatest effect, but this was not observed, as 1000 nmol amitriptyline had no effect beyond that of the 300 nmol dose. Furthermore, the enhanced appearance of adenosine following capsaicin or the augmentation in the presence of amitriptyline plus exogenous adenosine have no ready explanation in such a scheme.

4.5. Summary

The present study indicates that amitriptyline enhances extracellular tissue levels of adenosine in the rat hindpaw following local administration in vivo. This adenosine may result partially from the extracellular conversion of nucleotide and partially from inhibition of uptake. The enhanced tissue levels of adenosine are observed at doses of amitriptyline that produce peripheral antinociception and provide a plausible explanation for the methylxanthine-sensitivity of antinociception observed in several studies.

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