Hippocampal Noradrenaline Release in Awake, Freely Moving Rats Is Regulated by Alpha-2 Adrenoceptors but Not by Adenosine Receptors

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ABSTRACT
In this study, the ability of the nonselective adenosine receptor antagonist caffeine to influence the concentration of noradrenaline in the central nervous system was investigated, and its effects compared with those of alpha-2 adrenoceptor modulation. The technique of microdialysis in association with microbore high-performance liquid chromatography and electrochemical detection was used to measure the extracellular concentrations of noradrenaline in the hippocampus of awake, freely moving rats. Neither the oral administration of caffeine nor its local perfusion influenced the base-line hippocampal levels of noradrenaline. Furthermore, the levels of noradrenaline were not influenced by local perfusion of the selective adenosine A1 agonist N6-cyclopentyladenosine or by the selective adenosine A2 agonist CGS 21680. In contrast, the extracellular levels of noradrenaline could be increased by the perfusion of the selective alpha-2 adrenoceptor antagonist idazoxan and decreased by local perfusion of Ca2+-free phosphate buffered saline, a Na+-channel blocker, tetrodotoxin, or the selective alpha2-adrenoceptor agonist clonidine. The extracellular levels of noradrenaline were stimulated by the local perfusion of different concentrations of K+ (10–100 mmol/l). The K+-dependent increase in the extracellular levels of noradrenaline was potentiated by local perfusion of idazoxan and inhibited by local perfusion of clonidine. In contrast, neither the oral administration of caffeine nor its local perfusion influenced the K+-stimulated increases in hippocampal noradrenaline. Furthermore, local perfusion of N6-cyclopentyladenosine or CGS 21680 did not influence the K+-stimulated levels of noradrenaline either. These results indicate that base-line and K+-stimulated extra-cellular levels of noradrenaline in the hippocampus of awake, freely moving rats are regulated by alpha-2 adrenoceptors and not by adenosine receptors.

Caffeine and theophylline are popular drugs which possess a complex psychopharmacological profile (Blättig and Welzl, 1993). Structurally, they belong to a class of compounds known as methylated xanthines or methylxanthines (Rall, 1993). Although several mechanisms of action have been proposed to explain their physiological effects, antagonism of adenosine receptors is the most likely (Snyder et al., 1981). Adenosine receptors are classified into two subtypes based on their ability to regulate the accumulation of cyclic AMP: A1 receptors inhibit cyclic AMP accumulation, whereas A2 receptors stimulate it (Van Calker et al., 1979; Londos et al., 1980). Methylxanthines antagonize the effects of adenosine at both A1 and A2 receptors (Daly et al., 1983; Bruns et al., 1986).

Caffeine is a potent stimulant of the central nervous system. Many people who ingest caffeine claim to experience arousal characterized by reduced drowsiness and fatigue together with a more rapid and clearer flow of thought (Curatolo and Robertson, 1983; Rall, 1993). Until recently, however, the neurochemical basis for these effects was unclear. Now, two studies have provided evidence for a functional link between neuronal control of arousal, antagonism of adenosine A1 receptors and central cholinergic function (Rainnie et al., 1994; Carter et al., 1995). Not all the psychostimulant effects of caffeine can be regarded as positive. Tasks involving delicate muscle coordination and accurate timing may be adversely affected by the ingestion of caffeine (Curatolo and Robertson, 1983). Indeed, many of the motoric side effects of methylxanthines are similar to those induced by dopamine agonists, and there is evidence for a direct interaction between the striatal adenosine A2 receptor and the dopamine D2 receptor (Ferré et al., 1992, 1993). In addition to the aforementioned effects, caffeine also causes increased nervousness and anxiety particularly among psychiatric patients (Blättig and Welzl, 1993; Rall, 1993), a modest pressor effect on the cardiovascular system and increases in plasma catecholamines and sympathetic nerve activity (Mosqueda-Garcia et al., 1993; Rall, 1993). Preclinical studies investiga-
ing the physiological mechanisms of fear and anxiety have implicated the brain noradrenergic system as one of the main substrates for these effects (Charney et al., 1995). Furthermore, evidence suggests that changes in activity of noradrenergic neurons in the locus ceruleus accompany changes in behavioral state (Foote and Aston-Jones, 1995).

NA is widely distributed in the mammalian brain and noradrenergic fibers can be divided into two major pathways on the basis of their major axonal projections: the dorsal and ventral bundles (Weiner and Molinoff, 1994). The neurons of the dorsal bundle arise in the locus ceruleus, located in the upper pons region of the medulla, and send projections to the spinal cord, cerebellum, the entire cerebral cortex and hippocampus. The neurons of the ventral bundle of the subcere-uleus region send projections to the brainstem and hypothalamus. Pharmacological studies have demonstrated the existence of at least four distinct subtypes of adrenoceptor present in both the peripheral and central nervous systems: beta-1, beta-2, alpha-1, and alpha-2 adrenoceptors (Weiner and Molinoff, 1994). The presynaptic receptor which modulates noradrenaline synthesis and release is an alpha-2 adrenoceptor (Starke et al., 1989).

In vitro experiments with brain slices from hippocampus and cortex have shown that adenosine and several of its stable analogs dose-dependently inhibit [3H]NA release elicited by electrical field stimulation or K+; and that this inhibition can be antagonized by perfusion of methylxanthine adenosine antagonists (Harms et al., 1978; Fredholm et al., 1983; Jackisch et al., 1985; von Kügelgen et al., 1992). Evidence suggests that adenosine-induced inhibition of [3H]NA release in brain slices is mediated by adenosine A1 receptors (Jackisch et al., 1985; von Kügelgen et al., 1992). However, there have been few in vivo studies. Although one published study indicated that high doses of caffeine (100–200 mg/kg i.p.) altered regional utilization of monamines in mice, there have been no systematic studies which have investigated the effects of adenosine receptor blockade on the extracellular levels of NA in vivo, and compared them with the effects of alpha-2 adrenoceptor modulation.

Microdialysis is a technique that allows the continual perfusion of discrete brain areas in vivo with minimal exposure of the brain tissue to the perfusion medium (Ungerstedt, 1991). The technique has been used in the past in association with HPLC and electrochemical detection to determine the extracellular concentrations of NA in different brain areas of rats (Heureux et al., 1986; Abercrombie et al., 1988; Itoh et al., 1990; van Veldhuizen et al., 1990). In this study, we have used the technique of microdialysis to investigate the ability of caffeine to influence the extracellular concentration of NA in awake, freely moving rats, and to compare its effects with those of alpha-2 adrenoceptor modulation.

Materials and Methods

Animals. Male Wistar rats weighing 250 to 340 g were housed in cages in groups of 4 to 10. Water and dried laboratory food (Altromin®) from Altromin (Lage, Germany) were freely available. The cages were kept in air-conditioned rooms at a temperature of 22°C (range, 21–25°C) and a relative humidity of 55% (range, 55–70%); the lighting was set to a 12-h light-dark cycle (lights on at 6:00 A.M.).

Reagents. DPBS was purchased from Serva (Heidelberg, Germany) and had the following composition: NaCl, 137 mmol/l; Na2HPO4·2H2O, 5.6 mmol/l; Na2HPO4·H2O, 0.9 mmol/l; MgSO4·7H2O, 0.8 mmol/l; KH2PO4, 1.5 mmol/l; CaCl2, 0.9 mmol/l; KCl, 2.7 mmol/l, pH 6.0. NA and 5-HT were purchased from Sigma Chemie GmbH (Deisenhofen, Germany). CPA, adrenale, idaxoxan, nisoxetine, clonidine and CGS 21680 were obtained from Research Biochemicals Inc. (Natick, MA) and TTX was from Serva (Heidelberg, Germany). Acetoni-etrin (Lichrosolv®) was purchased from E. Merck (Darmstadt, Germany). Caffeine (anhydrous) was synthesized at Boehringer Ingelheim and conformed with the requirements of the European Pharmacopoeia, 2nd edition (1995). All other reagents were of at least reagent grade and purchased from reputable sources. Caffeine was prepared for oral administration by dissolving freshly each day in 0.5% methylcellulose solution and administered by gavage (5 ml/kg).

Microdialysis procedure. The procedure was carried out essentially as previously described (Carter et al., 1995). Male Wistar rats were anesthetized with sodium pentobarbitone (50 mg/kg i.p.) and a microdialysis probe (3-mm loop, CMA/12, CMA Microdialysis, Stockholm, Sweden) and guide cannulae were implanted into the lateral hippocampus with the stereotactic coordinates –3.6 mm rostral, +4.6 mm lateral and –7.0 mm ventral relative to the bregma and dura according to the atlas of Pellegrino et al. (1979). The probes were secured by means of a guide cannula, two small metal screws and a small amount of Technovit® cold curing resin from Kulzer (Wehrheim/Taunus, Germany). On the following day, approximately 18 to 20 h after implantation, the rat was placed in a freely moving system (CMA/120, CMA Microdialysis, Stockholm, Sweden) and the microdialysis probe was perfused (2 μl/min) with DPBS. Unless otherwise stated, the perfusate contained nisoxetine (0.1 mmol/l), a selective noradrenaline uptake inhibitor. Samples (40 μl) were collected every 20 min with an autosampler. The microdialysis probe was first perfused for 80 min before starting to collect samples for measuring. The next two to four samples were collected with an autosampler, and the concentration of NA was determined by HPLC. The mean of these values was defined as base-line levels (100%). Samples were collected every 20 min thereafter, and the amount of NA was measured to determine the effects of the various drugs. None of the pharmacological agents administered produced any overt changes in behavior of the animals.

Determination of NA by HPLC. NA in the samples was measured by a sensitive microbore HPLC system. It consisted of an L-6200 intelligent pump (Merck-Hitachi, Darmstadt, Germany), an LC-4C electrochemical detector (BAS, West Lafayette, IN) with a glassy carbon working electrode (700 mV), a Gynkotek DG-1300 (Gynkotek, Germering, Germany) on-line degasser and a Gilson 228 (Gilson, Villiers-le-Bel, France) autosampler. The injection volume was 5 μl. The column used was a SepStik® Unijet® (BAS, West Lafayette, IN) C18 polymeric column (100 × 1 mm) with a 5-μm particle size in association with a small guard column (14 × 1 mm) filled with the same material. The mobile phase was made by preparing 1 liter of a solution containing 60 mmol/l sodium acetate, 0.5 mmol/l ethylenedinitroltetraacetic acid and 5 mmol/l 1-decane sulfonic acid sodium salt. After the solution was adjusted to a pH of 6.0 with HCl (1 mol/l), 130 ml acetoni-etrin was added to give a final concentration of 11.5%. The mobile phase was then filtered (0.2 μm) and pumped at a flow rate of 100 μl/min. Purified water was prepared with a Millipore Milli-Q-system.

Data calculation and presentation. HPLC data were collected with a Nelson analytical series 900 interface from ESWE Analytik (Sinsheim, Germany). Peak integration was carried out with Turbochrom® software version 4.1 from Axel Semrau (Sprockhövel, Germany) on a personal computer. Peak identification and quantification were carried out with external standards. A two-way analysis of variance for repeated measures was applied to the logarithmically transformed serial determinations of NA. A Dunnett’s multiple comparison test was used to identify which values were significantly different from the base-line value at 40 min, or whether the total mean NA release after administration of caffeine differed from the release in the vehicle-control group. All values are expressed as the mean ± S.E.M., n ≥ 4; * denotes P < .05. Statistical calculations
were performed with SAS version 6.08 (SAS Institute, Cary, NC) and all graphs were plotted with SigmaPlot 3.02 for Windows® from Jandel Scientific (Jandel Scientific GmbH, Erkrath, Germany).

Results

The microbore HPLC system described in this paper achieved an excellent separation of NA, adrenaline and dopamine. The limit of quantitation for this assay was in the low nanomoles per liter range (10 fmol/40 μl dialysate) and the coefficient of variation for 16 consecutive injections of 5 μl of 10 nmol/l standard was 4.6%. Despite the high sensitivity of the microbore HPLC system, it was not possible to measure NA in the dialysate reproducibly. Therefore, the selective NA uptake inhibitor nisoxetine was added to the perfusate. The concentration of NA measured in the dialysate was directly proportional to the concentration of nisoxetine in the perfusate up to and including a concentration of 1 μmol/l nisoxetine was added to the perfusate to further increase in the base-line levels of NA. The mean concentration of NA in the presence of 0.1 μmol/l nisoxetine was 3.5 ± 1.0 nmol/l. Nisoxetine was added to the perfusate at a concentration of 0.1 μmol/l for all subsequent experiments.

Initially, I characterized the basal hippocampal NA release in the awake, freely moving rat by monitoring the effects of local perfusion with several agents with known effects on noradrenergic neurons. Removal of Ca²⁺ from the perfusate caused a significant reduction (up to 40%) in the basal extracellular levels of NA in the hippocampus (fig. 2A). In addition, inclusion of the Na⁺-channel blocker TTX (1 μmol/l) in the perfusate also significantly reduced (more than 50%) basal NA release (fig. 2A). Local perfusion of the alpha-2 adrenoceptor antagonist idazoxan (1 mmol/l) was associated with a rapid increase in the extracellular levels of NA (fig. 2B). In contrast, local perfusion of the alpha-2 adrenoceptor agonist clonidine (100 μmol/l) caused a significant decrease in the extracellular levels of NA (fig. 2B).

Oral administration of caffeine (30 mg/kg) failed to influence K⁺-stimulated levels of NA in the hippocampus of awake, freely moving rats (fig. 3A). However, there was a small, but insignificant increase in the extracellular levels of NA after administration of either caffeine or the vehicle control (0.5% methylcellulose) by gavage (fig. 3A). Base-line hippocampal levels of NA were not affected by local perfusion of the selective adenosine A₁ agonist CPA (1 μmol/l), by the selective adenosine A₂ agonist CGS 21680 (10 μmol/l) or by caffeine itself (1 mmol/l) as compared with control (fig. 3B).

Because neither oral administration of caffeine nor its local perfusion influenced the base-line levels of NA in the hippocampus of awake, freely moving rats, I decided to determine whether caffeine influenced K⁺-stimulated release of NA. Initially, I determined the effects of different concentrations of K⁺ in the perfusate on hippocampal NA release. Local perfusion of different concentrations of K⁺ (10–100 mmol/l) caused an increase in the extracellular levels of NA. The relative increase in the hippocampal levels of NA was not dependent on the concentration of K⁺ in the perfusate above 10 mmol/l. However, the time of onset of the response was: the higher the concentration of K⁺ in the perfusate, the quicker the maximum response was achieved (fig. 4A). Local perfusion of the selective alpha-2 adrenoceptor antagonist idazoxan (1 mmol/l) enhanced the K⁺-stimulated levels of NA, whereas local perfusion of the selective alpha-2 adrenoceptor agonist clonidine (100 μmol/l) depressed them (fig. 4B).

Oral administration of caffeine (30 mg/kg) failed to influence K⁺-stimulated levels of NA in the hippocampus of awake, freely moving rats (fig. 5A). Furthermore, K⁺-stimulated levels of hippocampal NA were not affected by local perfusion of the selective adenosine A₁ agonist CPA (1 μmol/l), by the selective adenosine A₂ agonist CGS 21680 (10 μmol/l) or by caffeine itself (1 mmol/l) as compared with control (fig. 5B).

Discussion

Microdialysis has been used in the past with classical HPLC techniques and electrochemical detection to determine

![Fig. 1. The effect of nisoxetine concentration on the extracellular level of NA in the hippocampus of awake, freely moving rats. The microdialysis probe was perfused with different concentrations of the selective NA uptake inhibitor nisoxetine (0.01–10 μmol/l) and the samples were collected and measured with a microbore HPLC system in association with electrochemical detection. The microbore HPLC measurement of NA within 5 min in the microdialysis samples (A) and the concentration of NA in the sample depended on the concentration of nisoxetine in the perfusate up to a concentration of 10 μmol/l (A, B).](image-url)
the extracellular concentrations of NA in different brain areas of rats (Abercrombie et al., 1988; Itoh et al., 1990; van Veldhuizen et al., 1990). The results of this study show that a microbore HPLC technique can also be applied to measurement of NA in brain dialysates. The benefits derived include increased sensitivity leading to smaller injection volumes, shortened analysis times and reduced use of organic mobile phase (Carter, 1994). Nevertheless, despite the excellent sensitivity of the microbore system, it was still necessary to include a selective NA uptake inhibitor, nisoxetine, in the perfusate to enhance base-line levels of NA. Nisoxetine appears to be much more effective as an inhibitor of NA uptake in the hippocampus than in the nucleus accumbens. A previous study has shown that concentrations of nisoxetine in excess of 40 μmol/l are required to increase base-line NA release 3-fold in the latter brain area (Li et al., 1996). Nisoxetine was used at a concentration of 0.1 μmol/l for all subsequent experiments because this was the minimum concentration necessary to obtain stable, reproducible levels of NA in the perfusate that were high enough to detect physiological and pharmacological decreases. Nevertheless, I considered it important to characterize this preparation carefully with different pharmacological and physiological agents of known actions.

The base-line extracellular concentrations of NA in the hippocampus of awake, freely moving rats were inhibited by local perfusion of a Ca²⁺-free DPBS or 1 μmol/l TTX (A). Local perfusion of an alpha-2 adrenoceptor antagonist idazoxan (1 μmol/l) resulted in a rapid and prolonged increase in extracellular levels of NA, whereas local perfusion of an alpha-2 agonist clonidine (100 μmol/l) caused a significant decrease (B). A two-way analysis of variance for repeated measures was applied to the logarithmically transformed values for the serial determination of NA, followed by a Dunnett’s multiple comparison test to identify which values were significantly different from the base-line value at 40 min. All values are expressed as the mean ± S.E.M., n ≥ 4; * denotes P < .05.

Fig. 2. Physiological and pharmacological characterization of basal extracellular release of NA in the hippocampus of awake, freely moving rats. Basal extracellular levels of NA were inhibited by local perfusion of a Ca²⁺-free DPBS or 1 μmol/l TTX (A). Local perfusion of an alpha-2 adrenoceptor antagonist idazoxan (1 μmol/l) resulted in a rapid and prolonged increase in extracellular levels of NA, whereas local perfusion of an alpha-2 agonist clonidine (100 μmol/l) caused a significant decrease (B). A two-way analysis of variance for repeated measures was applied to the logarithmically transformed values for the serial determination of NA, followed by a Dunnett’s multiple comparison test to identify which values were significantly different from the base-line value at 40 min. All values are expressed as the mean ± S.E.M., n ≥ 4; * denotes P < .05.

Fig. 3. The effect of various adenosine receptor agonists and antagonists on the base-line level of NA in the hippocampus of awake, freely moving rats. Oral administration of the nonselective adenosine receptor antagonist caffeine (30 mg/kg) or an equal volume of vehicle (0.5% methylcellulose) failed to influence base-line hippocampal levels of NA (A). Furthermore, local perfusion (striped bar) with the A₁ agonist CPA (1 μmol/l), the A₂ agonist CGS 21680, the nonselective adenosine receptor antagonist caffeine (1 μmol/l) or control also failed to influence base-line levels of NA (B). A two-way analysis of variance for repeated measures was applied to the logarithmically transformed values for the serial determination of NA, followed by a Dunnett’s multiple comparison test to identify which values were significantly different from the base-line value at 40 min. All values are expressed as the mean ± S.E.M., n ≥ 4; * denotes P < .05.
the extracellular levels of NA. Previous microdialysis studies have shown that addition of alpha-2 adrenoceptor antagonists, such as idazoxan or yohimbine, to the perfusate causes an increase in the extracellular levels of NA in the hypothalamus and cortex including the hippocampus (Heureux et al., 1986; Dennis et al., 1987; Abercrombie et al., 1988; Itoh et al., 1990; Thomas and Holman, 1991; van Veldhuizen et al., 1993, 1994). Noradrenergic neurons are thought to be under inhibitory feedback control by presynaptic alpha-2 adrenoceptors (Starke et al., 1989). Indeed, more recent microdialysis experiments with subtype selective alpha-2 adrenoceptor antagonists indicate that the presynaptic alpha-2A adrenoceptor modulating hippocampal NA release is the alpha-2D subtype (Kiss et al., 1995). Perfusion of the alpha-2 adrenoceptor agonist clonidine caused a significant decrease in the extracellular levels of NA in the hippocampus of awake, freely moving rats. Consequently, even in the presence of a NA uptake inhibitor and enhanced base-line levels of NA, there is still room to activate autoreceptors to reduce the release of NA. These findings confirm earlier findings where clonidine was either perfused through the microdialysis probe or administered systemically (Heureux et al., 1986; Abercrombie et al., 1988; van Veldhuizen et al., 1993).

Having characterized the present system as being under regulatory control of alpha-2 adrenoceptors, I investigated whether activation or antagonism of adenosine receptors influenced NA release. The dose of caffeine and concentrations of selective agonists used in this study were based on experience from a previous study (Carter et al., 1995). Here oral administration of 30 mg/kg caffeine produced large, long-lasting increases in acetylcholine release in the hippocampus of awake, freely moving rats. Contrary to my initial expectations, neither the oral administration of caffeine at this dose nor its local perfusion influenced the base-line hippocampal levels of NA. Furthermore, local perfusion (striped bars) with the A1 agonist CPA (1 μmol/l), the A2 agonist CGS 21680, the nonselective adenosine receptor antagonist caffeine (1 mmol/l) or control also failed to influence KCl-stimulated levels of NA (B). A two-way analysis of variance for repeated measures was applied to the logarithmically transformed values for the serial determination of NA, followed by a Dunnett’s multiple comparison test to identify which values were significantly different from the base-line value at 40 min. All values are expressed as the mean ± S.E.M., n ≥ 4; * denotes P < .05 for all experiments.

Fig. 4. Pharmacological characterization of KCl-stimulated NA release in the hippocampus of awake, freely moving rats. Local perfusion (striped bar) of different concentrations of KCl (10–100 mmol/l) significantly stimulated the extracellular levels of NA (A). The onset of the maximum increase of NA was dependent on the concentration of KCl. Local perfusion (striped bar) of the alpha-2 adrenoceptor antagonist idazoxan (1 mmol/l) potentiated the KCl-induced increase in hippocampal NA levels, whereas the alpha-2 adrenoceptor agonist clonidine (100 μmol/l) inhibited it (B). A two-way analysis of variance for repeated measures was applied to the logarithmically transformed values for the serial determination of NA, followed by a Dunnett’s multiple comparison test to identify which values were significantly different from the base-line value at 40 min. All values are expressed as the mean ± S.E.M., n ≥ 4; * denotes P < .05. For clarity the asterisks are not shown in A.

Fig. 5. The effects of various adenosine receptor agonists and antagonists on the KCl-stimulated increases in the levels of NA in the hippocampus of awake, freely moving rats. Oral administrations of caffeine (30 mg/kg) or an equal volume of vehicle control (0.5% methylcellulose) failed to influence KCl-stimulated levels of NA (A). Furthermore, local perfusion (striped bars) with the A1 agonist CPA (1 μmol/l), the A2 agonist CGS 21680, the nonselective adenosine receptor antagonist caffeine (1 mmol/l) or control also failed to influence KCl-stimulated levels of NA (B). A two-way analysis of variance for repeated measures was applied to the logarithmically transformed values for the serial determination of NA, followed by a Dunnett’s multiple comparison test to identify which values were significantly different from the base-line value at 40 min. All values are expressed as the mean ± S.E.M., n ≥ 4; * denotes P < .05 for all experiments.
ycellulose (5 ml/kg) by gavage, irrespective of whether it contained caffeine or not. Previous work has shown that mild stressors such as handling or tail pinching can cause small (approximately 50%), transient increases in base-line hippocampal levels of NA (Kalens et al., 1989; Cenci et al., 1992). Oral administration by gavage is also stressful for the animals and produces small increases in NA levels. This is not to be confused with a compound-induced effect. Under base-line conditions NA release does not appear to be regulated by adenosine receptors, despite the fact that the agents used have potent effects on acetylcholine release in the hippocampus of awake, freely moving rats at doses and concentrations similar to those used in this study (Carter et al., 1995).

At first sight, in vitro experiments with brain slices from different brain areas seem to contradict the present study. Rat hippocampal slices can be induced to release [3H]NA by electrical field stimulation and this release can be inhibited by perfusion of various adenosine agonists (Fredholm et al., 1983; Jackisch et al., 1985). Adenosine and related nucleotides also inhibit the release of [3H]NA from rat cortical slices which had been stimulated by the perfusion of depolarizing concentrations of K+ (Harms et al., 1978) and from rabbit cortical slices stimulated with electrical field pulses (von Kügelgen et al., 1992). However, in all of these experiments the release of exogenously added [3H]NA was measured and the absolute effects of adenosine or its analogues were quite small. For example, adenosine or a stable analog, N6-phenylisopropyl-adenosine, inhibited [3H]NA release by a maximum of only 30 to 40% (Harms et al., 1978; Fredholm et al., 1983). Finally, all of these experiments involved the artificial stimulation of [3H]NA release with electrical field stimulation or K+. Anesthesia might also play a role. Given the long half-life of the anesthetic agent used in this study (sodium pentobarbitone), I cannot exclude the possibility that this may also account for some of the differences between my in vivo findings and published in vitro results, despite waiting 18 to 20 h after anesthesia before performing the experiments.

The results of this study show that perfusion of different concentrations of K+ through the microdialysis probe implanted in the hippocampus also caused an increase in the endogenous levels of NA. At concentrations beginning with 30 mmol/l the speed of onset of NA release was dependent on the concentration of K+ in the perfusate. Previous work has shown that perfusion of K+ through a microdialysis probe located in the anterior hypothalamus or electrical stimulation of ascending noradrenergic pathways projecting to the cortex caused an increase in the levels of NA in the target areas (Heureux et al., 1986; Badoer et al., 1989). However no experiments have been performed, to my knowledge, which show whether the stimulated levels of NA in the brains of awake, freely moving rats are regulated by alpha-2 adrenoceptors or adenosine receptors. The results of the present study demonstrate that the K+-stimulated levels of NA in the hippocampus are indeed regulated by alpha-2 adrenoceptors. Idazoxan, an alpha-2 adrenoceptor antagonist, enhanced K+-stimulated levels of NA, whereas clonidine, an alpha-2 adrenoceptor agonist inhibited these levels. Nevertheless, despite this regulatory control by autoreceptors, there was no effect of caffeine on K+-stimulated levels of NA in the hippocampus irrespective of whether caffeine was administered orally or directly perfused through the microdialysis probe. Furthermore, perfusion of the selective adenosine A1 and A2 receptor antagonists, CPA and CGS 21680, respectively, through the microdialysis probe did not influence the K+-stimulated levels of NA, despite being used at concentrations which are known to counteract the effects of orally administered caffeine on acetylcholine release (Carter et al., 1995).

The hippocampus is one of the best characterized cortical structures and its activity is influenced by a variety of different neurotransmitters, including acetylcholine, γ-aminobutyric acid, histamine, NA and adenosine (Brown and Zador, 1990). There is evidence for both a convergence and divergence of neurotransmitter action in cortical structures. Individual neurons in the hippocampus can respond to more than one putative neurotransmitter with the same ionic response, and yet, virtually all neurotransmitters act as at least two distinct receptor subtypes coupled to different ion channels in the same cell (Nicoll, 1988). For instance, activation of beta adrenergic receptors in pyramidal cells leads to a decrease in potassium current, whereas activation of adenosine receptors leads to an increase in potassium current (McCormick and Williamson, 1989). This convergence and divergence of neurotransmitter action complicates our understanding of neuronal activity in vivo. In the natural in vivo state it is likely that cells in any given brain structure are under a constant influence of a dynamically changing array of neuroactive substances (McCormick and Williamson, 1989). It is not surprising, therefore, perhaps that effects of adenosine on NA release in various in vitro slice systems cannot necessarily be reproduced exactly in the in vivo situation.

In summary, the results of this study show that base-line and K+-stimulated extracellular levels of NA in the hippocampus of awake, freely moving rats are regulated by alpha-2 adrenoceptors and not by adenosine receptors. Therefore, increases in nervousness and anxiety induced by the administration of caffeine are probably not related to an interaction of adenosine receptors and the noradrenergic system in the hippocampus.

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References


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