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The Effect of Long Term Caffeine Treatment on Hypoxic-Ischemic Brain Damage in the Neonate

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ABSTRACT

There is considerable concern over the widespread use of caffeine during and after pregnancy. We have therefore examined the effect of perinatal caffeine use on the vulnerability of the immature brain to hypoxic ischemia (HI). Rat pups were exposed to caffeine during the first 7 d after birth by addition of a low or a high dose (0.3 or 0.8 g/L) of caffeine to the drinking water of their dams. At 7 d the pups were exposed to unilateral carotid occlusion + exposure to 7.70% oxygen for 100 min. The extent of HI brain damage was evaluated 2 wk after the insult. The effects of caffeine on A_1 and A_{2a} receptors, A_1 mRNA and A_{2a} mRNA, were examined by receptor autoradiography and *in situ* hybridization. Caffeine, theobromine, theophylline, and paraxanthine were analyzed in plasma of separate animals. Exposure to caffeine reduced HI brain damage from 40.3 ± 3.2% in controls to 29.8 ± 4.0% ($p < 0.05$) in low dose and 33.7 ± 3.9% (NS) in the high dose group. The A_1 receptor density measured as [^{3}H]-1,3-dipropyl-8-cyclopentyl xanthine ([^{3}H]-DPCPX) binding was not significantly affected after low dose caffeine but increased in the brain of rat pups in the high dose group. The A_{2a} receptor density measured as [^{3}H]-2[*p*-(2-carbonylethyl)-phenethylamino]-5′-*N*-ethylcarboxamidoadenosine ([^{3}H]-CGS 21680) binding and the expression of A_1 mRNA and A_{2a} mRNA were not altered by caffeine treatment. In conclusion, low dose caffeine exposure (plasma levels corresponding to umbilical cord plasma in newborns of coffee-consuming mothers) reduced HI brain damage by 30% in 7-d-old rats. This ameliorating effect could not be accounted for by up-regulation of adenosine receptors. (***Pediatr Res* 38: 312–318, 1995**)

Abbreviations

HI, hypoxic ischemia
DPCPX, 1,3-dipropyl-8-cyclopentyl xanthine
CGS 21680, 2[*p*-(2-carbonylethyl)-phenethylamino]-5′-*N*-ethylcarboxamidoadenosine

Caffeine is widely consumed by women during pregnancy and immediately thereafter (1) and it is used, together with its metabolite theophylline, in the treatment of premature apnea (2). Furthermore, the fetus and the newborn become exposed because caffeine crosses the placenta (3, 4) and diffuses into the breast milk (5). Caffeine affects several systems in the body, *e.g.* the renal, respiratory, cardiovascular (6), gastrointestinal, and the CNS (7, 8). Caffeine is metabolized in the liver (9), and the metabolites are then excreted in the urine. The metabolism is much slower in neonates than in adults: $t_{1/2}$ in infants is 50–103 h (7, 10, 11) and $t_{1/2}$ in adults is 2–6 h (7, 12). Several recent reports have raised concern about the safety of caffeine use during and after pregnancy (13).

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Caffeine is a receptor antagonist for the endogenous nucleoside adenosine. Adenosine influences various transmitter systems in the CNS: noradrenaline, dopamine, serotonin, acetylcholine, γ-aminobutyrate, and glutamate (14). Adenosine also has neuroprotective actions during ischemia (15–20). The extracellular concentration of adenosine increases rapidly during ischemia (21, 22), and the number of adenosine receptors decreases promptly (23, 24). In the adult brain, chronic caffeine treatment, which leads to up-regulation of adenosine receptors, reduces ischemic damage (25), whereas acute exposure (receptor antagonistic effect) increases ischemic damage (26). The purpose of this study was to extend present knowledge to the neonatal setting, considering the common situation of fetal and neonatal caffeine exposure (1). Adenosine receptors appear and are functional at an early ontogenetic age (27, 28). There are four types of adenosine receptors, A_1, A_{2a}, A_{2b}, and A_3, all of which are expressed in the brain (29). A_1 and A_{2a} receptors are the most likely targets for caffeine actions. Exposure of neonatal rats to high amounts of caffeine given by gavage during postnatal d 2–6 increases the binding and effect of an A_1

receptor agonist (30). We investigated the effect of treating the pregnant and lactating female rat with caffeine on the extent of HI brain damage in their offspring. In addition, binding of the A_1 receptor ligand [^{3}H]-DPCPX and the A_{2a} receptor ligand [^{3}H]-CGS 21680, and adenosine receptor A_1 and A_{2a} mRNA were examined, because earlier studies have suggested that caffeine-induced neuroprotection is explained by up-regulation of adenosine (A_1) receptors (25).

METHODS

Experimental procedures. Fifteen pregnant Wistar F rats and their litters were used. Anhydrous caffeine was obtained from Sigma Chemical Co. (St. Louis, MO). The experiments were designed to allow the dam to adapt to caffeine exposure before the start of the experimental period. Therefore a prenatal dose was included in the drinking water in different concentrations (0, 0.3, and 0.8 g/L) throughout gestational d 8–21. Postnatally, pups were exchanged between three dams (receiving 0, 0.3, and 0.8 g/L) so that 1/3 stayed with their biologic dam and 2/3 were divided between the remaining two. Dams were given 0 (control), 0.3 (low dose), and 0.8 (high dose) g/L of caffeine, respectively, during postnatal d 1–7. The dams accepted and fed pups from other litters. The daily intake of water was measured in all groups.

In the morning of d 7 the dams were given water without caffeine and the pups were exposed to HI as follows. The pups were anesthetized with halothane (2.5–3.0% for induction and 1.0–1.5% for maintenance) in a mixture of nitrous oxide and oxygen (1:1). The left common carotid artery was dissected and cut between double ligatures of silk sutures (6–0). The duration of anesthesia was <10 min. After the surgical procedure, the wounds were infiltrated with a local anesthetic. The pups were left to recover for at least 1 h. The litters were then placed in a chamber perfused with a humidified gas mixture (7.70 ± 0.01% oxygen in nitrogen) for 100 min. The temperature in the gas chamber was kept at 36.5 ± 0.2°C. After hypoxic exposure the pups were returned to their biologic dams and allowed to recover without caffeine treatment until postnatal d 21. All animal experiments were approved by the Ethical Committee of Göteborg (no. 131-93).

Evaluation of brain damage. At postnatal d 21 the pups were anesthetized with thiopental, and the brains were extracted. The brainstem and cerebellum were removed from the forebrain. The two cerebral hemispheres were separated and weighed on a high precision balance (Mettler Instruments AG, Greifensee, Switzerland, sensitivity ± 0.1 mg). The brain damage was expressed as ipsilateral hemisphere weight deficit as percent of the contralateral hemisphere (31). A satisfactory correlation has been shown in earlier studies between brain weight and other measures of injury (31–35).

Evaluation of plasma concentration of caffeine. Three biologic litters, *i.e.* the pups born to the same dam, were killed on postnatal d 7 without exposure to HI. After decapitation, blood was collected in heparinized plastic tubes and centrifuged. The concentration of caffeine and the caffeine metabolites theophylline, theobromine, and paraxanthine in plasma were analyzed immediately after caffeine withdrawal and 5 h after, corresponding to peak plasma levels and plasma levels at the time of hypoxic exposure. The HPLC system used did not separate theophylline and paraxanthine well, and the results are presented as the sum of the two metabolites.

Sections. Brains from the three biologic litters mentioned above were dissected and frozen in dry ice-chilled dimethylbutane. Evaluation of [^{3}H]-DPCPX binding, [^{3}H]-CGS 21680 binding, and adenosine receptor mRNA was done on coronal sections cut with a Leitz cryostat at the following anterior to posterior levels: +7.5, +6.5, +5.6, +4.4, +2.6, +1.6, +1.2, and +0.8 mm from a plane 4.1 mm posterior to the bregma. Sections 10 μm thick were thaw-mounted on gelatin-coated slides for quantitative receptor autoradiography. Sections 14 μm thick were thaw-mounted on poly-L-lysine (50 μg/mL)-coated slides for *in situ* hybridization.

Receptor autoradiography. Sections were preincubated in 170 mM Tris-HCl buffer containing 1 mM EDTA and 2 U/mL adenosine deaminase at 37°C for 30 min. To study binding to A_1 receptors the protocol described by Parkinson and Fredholm (36) was followed. Sections were washed twice for 10 min at 23°C in 170 mM Tris-HCl buffer containing 1 mM $MgCl_2$. Incubations were performed for 2 h at 23°C in Tris-HCl buffer containing 0.5 mM [^{3}H]-DPCPX (120 C_i/mmol, DuPont, Sweden), 2 U/mL adenosine deaminase, and 1 mM $MgCl_2$. Nonspecific binding was defined by 100 μM (*R*)-phenylisopropyladenosine. The experiments were carried out in the presence or absence of 100 μM GTP to convert all receptors to the low affinity state for agonists and in that way removing "cryptically" bound endogenous adenosine (36). Sections were then washed twice for 5 min each in ice-cold Tris-HCl, dipped quickly three times in ice-cold distilled water, and dried at 4°C over a strong fan. Films were apposed to the dried sections for 3 wk.

To study binding to adenosine A_{2a} receptors the procedure described by Johansson *et al.* (37) was followed. After preincubation sections were washed twice for 10 min at 23°C in 170 mM Tris-HCl buffer containing 10 mM $MgCl_2$. Incubations were performed for 120 min at room temperature in Tris-HCl buffer containing 10 nM [^{3}H]-CGS 21680 (43 C_i/mmol, DuPont), 2 U/mL adenosine deaminase, and 10 mM $MgCl_2$. Nonspecific binding was defined by 20 μM 2-chloroadenosine (Sigma Chemical Co.). The sections were washed twice for 5 min in ice-cold Tris-HCl, dipped quickly twice in ice-cold distilled water, and dried at 4°C over a strong fan. The sections were apposed to film for 5 wk.

In situ hybridization for A_1 and A_{2a} receptor mRNA. The 48-mer A_1 adenosine receptor probe was complementary to nucleotides 985-1032 of the rat A_1 receptor (37). The 44-mer A_{2a} probe was complementary to nucleotides 916–959 of the dog RDC8 cDNA (37). The oligodeoxyribonucleotides were radiolabeled using terminal deoxyribonucleotidyl transferase (Amersham Corp., UK) and ^{35}S-dATP (Amersham) to a specific activity of about 10 cpm/μg. Slide-mounted sections were hybridized in a cocktail containing 50% formamide (Baker, Sanford, ME), 4 × SSC, 1 × Denhardt's solution, 1% Sarkosyl, 0.02 M $NaPO_4$ (pH 7.0), 10% dextran sulfate, 0.5 mg/mL yeast tRNA (Sigma Chemical Co.), 0.06 M dithiotreitol, 0.1 mg/mL sheared salmon sperm DNA, and 10 cpm/mL probe.

After hybridization for 16 h at 42°C the sections were washed four times for 15 min each in 1 × sodium chloride-sodium citrate at 55°C (A_1 probe) or 45°C (A_{2a} probe), then dipped briefly in water, 60%, 95%, and 99.5% ethanol and air-dried. Finally the sections were apposed to Hyperfilm β-max from Amersham for 1–4 wk.

Statistics. Factorial Anova with Fisher correction was used for statistical analyses of brain damage evaluation. Differences in mortality were evaluated with χ^2 and Yates' correction. Independent *t* test and multivariate analysis of variance in the Systat Inc. (Evanston, IL) program were used for analyzing the results from quantitative receptor autoradiography and *in situ* hybridization. Statistical significance was expressed as $^*p < 0.05$, $^{**}p < 0.01$, and $^{***}p < 0.001$.

RESULTS

Effects of prenatal exposure to caffeine. The experiments were designed to evaluate the effects of *post*natal exposure to caffeine. The purpose of the *pre*natal exposure was merely to adapt the dams to caffeine. Prenatal exposure to caffeine may also affect HI brain damage. To avoid this confounding influence, postnatal treatment groups were composed of similar number of pups from each of the three prenatal exposure groups. Retrospective analysis showed that the brain damage was not different in the low dose prenatal exposure group (30.4 ± 1.9% weight deficit of the contralateral hemisphere) compared with the control group (30.5 ± 2.1%). However, there was a tendency to more extensive brain damage in pups prenatally exposed to high dose caffeine than prenatal control pups (45.2 ± 1.1% and 30.5 ± 2.1%, respectively, NS) (Table 1). Moreover, dams in the high dose group appeared stressed, and their pups were dehydrated, growth retarded, and suffered from a higher mortality (Table 1).

General comparison between the postnatal exposure groups. The mortality was low (0–2%) after HI in the control and caffeine-treated groups (Table 2). The body weight and contralateral hemispheric weight at the time of sacrifice and daily water intake were also similar in the low dose and the control group (Table 2). On the contrary, in the high dose group the body weight and the weight of the contralateral hemisphere tended to be lower (NS) and the water intake was lower ($p < 0.001$).

The mean daily intake of caffeine pre- and postnatally by the dams in the low dose group was 8.8 mg and 16.5 mg in the high dose group. Peak plasma concentration of caffeine in the pups was 0.26 ± 0.031 mg/L (mean ± SEM) in the low dose group and 0.014 ± 0.0078 mg/L in the control group. Peak plasma concentration of theophylline and paraxanthine was 0.58 ± 0.037 mg/L (mean ± SEM) in the low dose group and 0.039 ± 0.010 mg/L in the control group. Plasma concentrations of caffeine, theobromine, theophylline, and paraxanthine at the time of HI are given in Table 3.

Table 1. *Data for the three prenatal exposure groups*

Measurement	Group		
	Control (n = 37)*	0.3 g/L (n = 54)	0.8 g/L (n = 56)
Brain damage (%)	30.5 ± 4.4†	30.4 ± 3.5	45.2 ± 2.4
Body weight at birth (g)	6.1 ± 0.1	6.0 ± 0.1	5.0 ± 0.6
Weight of contralateral hemisphere (mg)	520.8 ± 8.6	537.0 ± 5.5	518.2 ± 5.3
Mortality day 0–21 (%)	21.6	20.4	48.2

* n = number of animals.
† Mean ± SEM.

Table 2. *Data for the three postnatal exposure groups*

Measurement	Group		
	Control (n = 39)*	0.3 g/L (n = 50)	0.8 g/L (n = 50)
Brain damage (%)	40.3 ± 3.2†	29.8 ± 4.0	33.7 ± 3.9
Body weight at 3 weeks (g)	27.6 ± 0.9	28.7 ± 0.8	23.9 ± 1.0
Weight of contralateral hemisphere (mg)	533.5 ± 7.2	533.0 ± 5.1	506.3 ± 7.5
Daily intake of water (ml/day and 10 pups)	29.7 ± 1.4	29.2 ± 0.5	20.7 ± 0.9***
Mortality day 7–21 (%) after HI insult	0	2.0	2.0

* n = number of animals.
† Mean ± SEM.
*** $p < 0.001$.

Brain damage in the postnatal exposure groups. Two weeks after HI, infarction and selective neuronal necrosis occurred in the cerebral cortex, thalamus, hippocampus, and striatum of the left hemisphere (ipsilateral hemisphere) (Fig. 1). Brain damage, >5% weight deficit of the ipsi- compared with contralateral hemisphere, developed in 96% of the control pups, *i.e.* 4% devoid of damage, compared with 27% in the low dose group and 17% in the high dose group. The mean brain damage amounted to 40.3 ± 3.2% in the control, 29.8 ± 4.0% in the low dose and 33.7 ± 1.7% in the high dose group, *i.e.* the brain injury was reduced by 30% ($p < 0.05$) in the pups treated with low dose caffeine compared with controls (Fig. 2). According to the above mentioned, high dose caffeine (pre- and postnatally) severely affected the health of the pups. However, even if all the pups prenatally exposed to high dose caffeine were excluded, there was a 38% reduction ($p < 0.05$) of brain damage in the low dose group (24.1 ± 4.7%) compared with control (38.6 ± 3.9%).

Changes in adenosine receptors. There was a high density of [^{3}H]-DPCPX binding in the cortex and in hippocampus, as expected. The binding in absence of GTP was lower than that observed in the presence of GTP in cortex (Table 4) and in hippocampus (not shown), in agreement with previous findings in adult animals (36). Optical densities were measured in hippocampus regions CA1 and CA3, dentate gyrus, and in

Table 3. *Plasma concentrations of methylxanthines in pups at time for HI*

Methylxanthine (mg/L)	Group		
	Control	0.3 g/L	0.8 g/L
Caffeine	0.000 ± 0.00*	0.22 ± 0.054	0.64 ± 0.00
Theophylline and Paraxanthine	0.000 ± 0.00	0.45 ± 0.099	1.09 ± 0.031
Theobromine	0.021 ± 0.00	0.42 ± 0.11	0.98 ± 0.00

The different doses indicate postnatal exposure groups.
* Mean ± SEM.

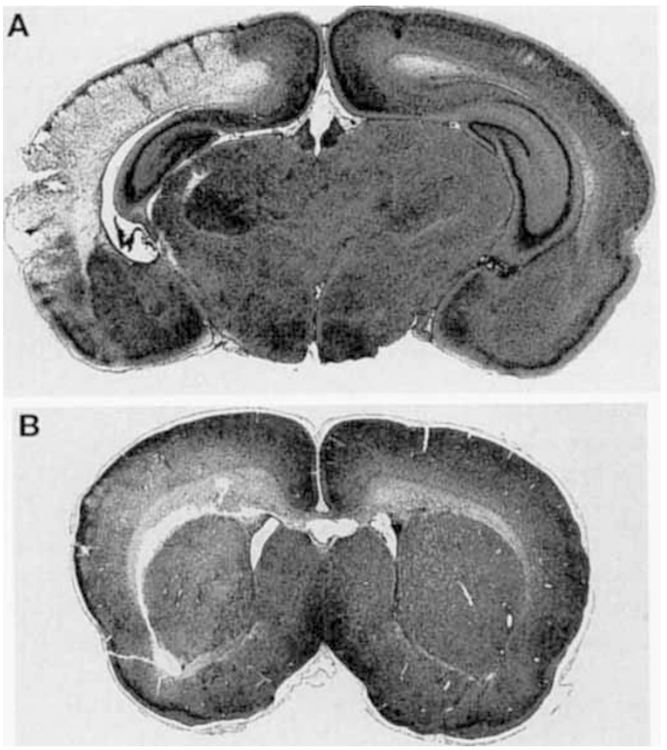

Figure 1. The distribution of the brain damage is shown by hematoxylin-eosin staining at postnatal d 21. Shown is the infarction and selective neuronal necrosis in the (*A*) cerebral cortex, thalamus, and hippocampus and (*B*) striatum of the left hemisphere.

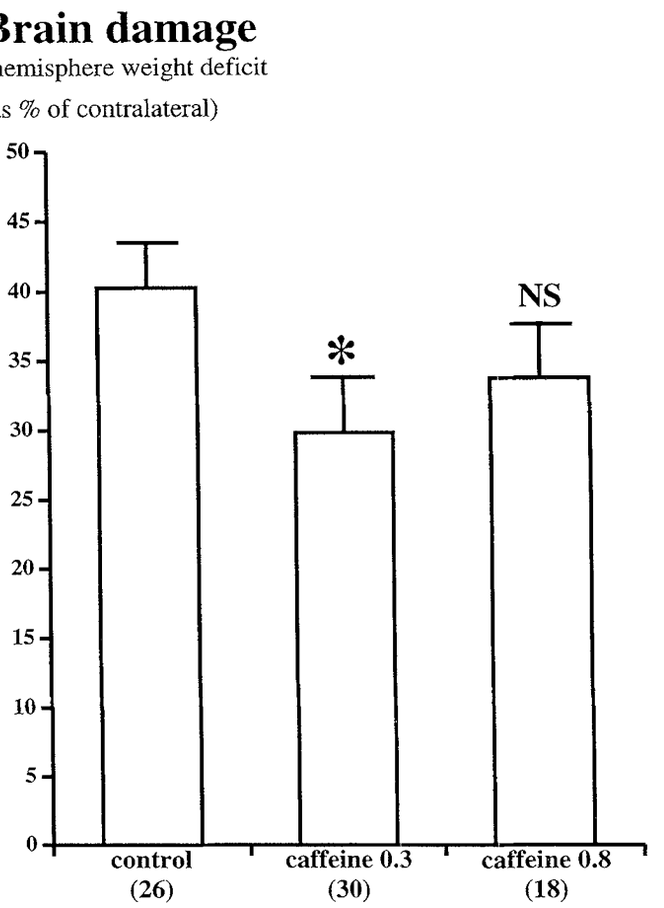

Figure 2. The effect of caffeine (0.3 or 0.8 g/L) on the degree of brain damage. Brain injury was evaluated by weighing the brains 2 wk after HI, and brain damage was expressed from the ipsilateral hemisphere weight deficit as a percentage of contralateral hemisphere weight. Values are set as mean ± SEM.

frontal and parietal cortex. Caffeine-treated rat pups showed a similar amount of [^{3}H]-DPCPX binding to CA1 and CA3 regions of the hippocampus as their controls (Table 5). In the cortex, the [^{3}H]-DPCPX binding was not significantly affected by caffeine treatment either when GTP-treated or untreated slices were examined (Table 4). However, in both groups there was a tendency toward increased binding, and when sections studied in the presence and absence of GTP were pooled, there was a significant difference between controls and pups in the high dose group ($p < 0.05$). Pups in the low dose group did not exhibit increased [^{3}H]-DPCPX binding (Fig. 3*A*).

A_1 mRNA was distributed mainly in the cortex and in hippocampus as shown earlier (37). The amounts in hippocampus CA1, CA3, dentate gyrus, and cortex were quantified. No significant differences between groups were seen in either region (Fig. 3*B* shows the results from cortex).

Adenosine A_{2a} receptors were studied by [^{3}H]-CGS 21680 binding. This ligand is an agonist, and therefore experiments in the presence and absence of GTP were less meaningful. Because these binding sites as well as the corresponding mRNA are enriched in the striatum (37), the measurements were confined to this region. As seen in Fig. 3*C* there were no major changes in striatal A_{2a} receptors. There were similarly no differences in A_{2a} receptor mRNA between treatments (Fig. 3*D*).

Table 4. *Binding of [^{3}H]-DPCPX to cortical adenosine A_1 receptors after treatment with different doses of caffeine postnatally*

	Postnatal treatment group		
	Control (n = 6)*	0.3 g/L (n = 10)	0.8 g/L (n = 4)
[^{3}H]-DPCPX binding + GTP	42.9 ± 5.8†	49.2 ± 2.2	55.6 ± 4.1
[^{3}H]-DPCPX binding − GTP	27.6 ± 2.3	32.7 ± 2.4	41.2 ± 3.9
Change from controls + GTP		+14.7%	+29.6%
Change from controls − GTP		+18.5%	+49.3%

Results are from animals prenatally exposed to 0.3 g/L caffeine in the drinking water. For other prenatal treatment, see Figure 3. Optical densities were converted to fmol/mg with microscales. The results are combined measurements from two levels (+6.5 and +2.5 mm from bregma, anterior to posterior).

* n = number of experiments.

† Mean ± SEM.

DISCUSSION

The published reports of prevention of ischemic damage in adults by adenosine receptor agonists and uptake inhibitors have led to the conclusion that adenosine may act as a protective agent (15, 38, 39). Much less is known about the situation in neonatal animals. Studies of neonatal HI have shown a reduction of brain damage with the adenosine uptake inhibitor propentofylline (40). However, propentofylline has effects that might be unrelated to adenosine, *e.g.* inhibition of oxygen free radicals and Ca^{2+} homeostasis, which could be relevant (41). The aim of the present study was to evaluate the role of

Table 5. *Binding of [^{3}H]-DPCPX to hippocampal adenosine A_1 receptors after treatment with different doses of caffeine postnatally*

	Postnatal treatment group		
	Control (n = 3)*	0.3 g/L (n = 5)	0.8 g/L (n = 5)
CA1	56.3 ± 3.0†	56.9 ± 3.9	68.3 ± 6.9
CA3	79.9 ± 7.1	70.1 ± 7.4	73.4 ± 2.4

Results are from animals prenatally exposed to 0.3 g/L caffeine in the drinking water. Similar results were obtained in the animals that had no prenatal caffeine exposure, but the number of observations in this group was smaller and is therefore not shown. Optical densities were converted to fmol/mg with microscales. The results are measurements at a level +1.6 mm from bregma (anterior to posterior). Results obtained only in the presence of GTP are shown.

* n = number of animals.

† Mean ± SEM.

adenosine receptors further with respect to HI brain damage by chronic exposure to caffeine before the insult.

The major finding was a reduction of brain damage in both groups treated with caffeine compared with the control group—a reduction that was significant for the low dose group (30%) (Fig. 2). Considering that caffeine is a weak, nonselective antagonist for adenosine receptors (12) and that the extracellular levels of endogenous adenosine during ischemia are high (22), the remaining extracellular levels of caffeine are expected to possess a negligible effect on adenosine receptors during HI. This is particularly true in the low dose group. In the group reared by dams fed 0.8 g/L caffeine, some receptor antagonism may occur. It is possible that this could contribute to the smaller beneficial effect in this high dose group.

The control and the low dose groups were similar with respect to confounding factors recorded (Table 2). This indicates that the *low* dosage of caffeine did not affect the growth of the body or the weight gain of the contralateral hemisphere. In contrast, the groups exposed to the *high* caffeine concentration were different (Table 2). These dams were hyperactive and the weight gain of the pups was deficient. These results correspond to earlier clinical studies on growth retardation in infants exposed to high concentrations of caffeine (1, 13). There was even a tendency toward more extensive brain damage after prenatal high dose of caffeine compared with controls. Due to the multiple side effects of caffeine this trend is difficult to interpret and may well be secondary to dehydration and/or malnutrition (42, 43).

Because the only known effect of caffeine in the concentration achieved here is blockade of adenosine receptors and because adenosine appears to be cerebroprotective, the mechanism behind the protection after long term caffeine treatment is obscure. In a study on gerbils long term caffeine treatment was associated with an up-regulation of adenosine A_1 receptors (25). However, the reduction of brain damage in the low dose group had no clear correlation with changes in A_1 receptors or in A_1 receptor mRNA. The group of neonatal pups exposed to low dose caffeine in breast milk showed no differences in [^{3}H]-DPCPX binding or adenosine receptor A_1 mRNA levels compared with unexposed animals. Our failure to demonstrate any change in adenosine receptors is likely to be related to the dose of caffeine used. In studies where up-regulation has been found after oral caffeine treatment, a plasma concentration of about 20 mg/L was achieved (37). We used doses which gave plasma concentrations in the 0.2–1 mg/L range. The present data are in complete agreement with results in mice where oral treatment with low doses of caffeine, producing plasma concentrations of 0.2–1 mg/L, had marked adaptive effects that were not associated with any change in receptor binding (44). Thus we conclude that the effects observed in this study are unlikely to be due to an *increased* transmission through A_1 receptors.

The reduction of brain damage in our model also cannot be related to influence of the A_{2a} receptors. The A_{2a} receptors are predominantly located on neurons in the dopamine rich regions of the brain and not in the cortical regions most affected by the ischemic damage (45). Furthermore, there were no major changes in A_{2a} ligand binding or in A_{2a} receptor mRNA levels.

Instead our working hypothesis is that caffeine, by blocking actions of endogenous adenosine, alters transmission in, *e.g.* glutamate-related and γ-aminobutyric acid-related pathways and that this induces adaptive changes that eventually may prove beneficial. It is known that caffeine treatment does induce adaptive changes in several types of receptors (46). Several of the affected transmitters (excitatory amino acids, γ-aminobutyric acid) (32–34, 47) have been implicated in cerebroprotection. It is also known that acute, as well as chronic, administration of caffeine induces changes in several immediate early genes, including c-*fos* (48). Because the product of immediate early genes acts as a transcription factor, several consequent changes can be anticipated. Irrespective of what the precise mechanism proves to be, it is likely that the cerebroprotective effect of long term caffeine treatment can be related to changes in susceptibility to seizures, spreading depression, or other forms of hyperexcitation, whereas acute administration of caffeine has the opposite effect (44).

Caffeine exposure to neonates may be clinically important. We have concentrated on a rat pup model that mimics the common situation of a pregnant or just delivered mother who drinks coffee and therefore passively exposes her fetus or child to caffeine. The developmental age of 7-d-old rats with respect to brain maturation corresponds to a near term human fetus (49). In our study the plasma concentrations of caffeine were similar to those seen in umbilical cord plasma in newborns of mothers consuming coffee (0.5–2 mg/L) (7). The plasma concentrations also resemble the levels in infant serum in studies with breast-fed infants and caffeine-exposed mothers (5, 11). In spite of these circumstances, extrapolation from rat to man is difficult, and the net effect of caffeine exposure may be different in a clinical setting. Nevertheless, the present finding that low dose administration of caffeine reduced ischemic brain damage is intriguing, and studies on pregnant women with different coffee consumption or newborns receiving caffeine as a treatment for apnea may be warranted. The importance of further investigation is also indicated by the tendencies toward an increased distress of pups born to dams that consumed a higher dose of caffeine.

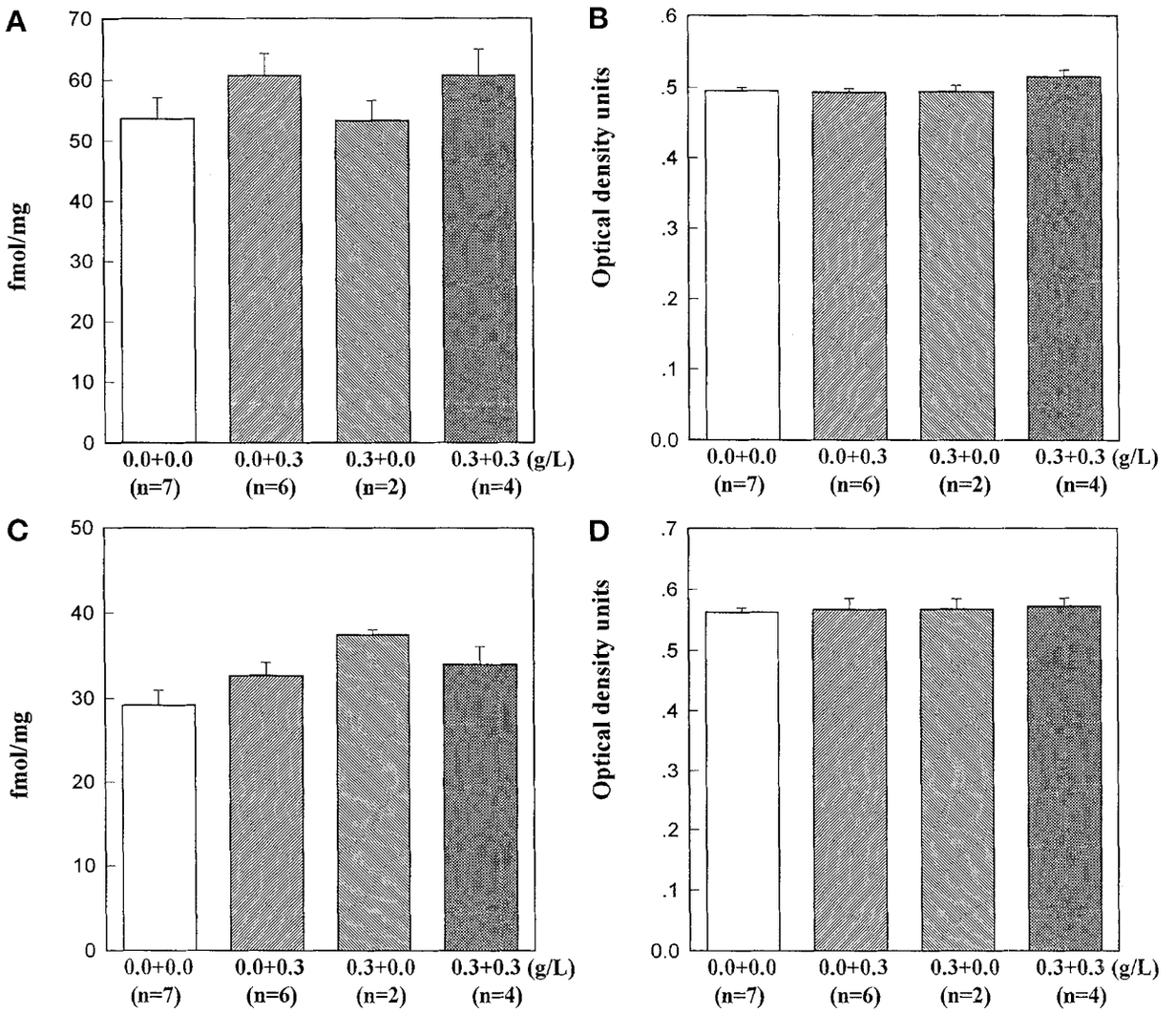

Figure 3. Adenosine A_1 and A_{2a} receptor binding and mRNA expression in rat pups exposed to caffeine, pre- and postnatally. Measurements were made at +1.6 mm (cortex) and +4.4 mm (striatum) from bregma (anterior to posterior). For binding quantification, OD was converted to fmol/mg with microscales. Values are set as mean ± SEM. Treatments are shown along the *x* axis where the first figure indicates the prenatal exposure and the second figure indicates the postnatal exposure. Number of measurements indicated in parentheses. (*A*) [^{3}H]-DPCPX binding in cortex; (*B*) adenosine receptor A_1 mRNA in cortex; (*C*) [^{3}H]-CGS 21680 binding in striatum; and (*D*) adenosine receptor A_{2a} mRNA in striatum.

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