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Review

The effects of prenatal exposure to methylxanthines

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Abstract

This review discusses epidemiology and laboratory studies on the effects of prenatal methylxanthine administration on some systems developing organisms. They are mainly absorbed from coffee, tea and cocoa products such as cola beverages and chocolate bars.

Prenatal methylxanthine exposure can induce several unfavourables changes in the developing organism, which are persistent even in later phases of life. Based on results obtained from animal studies, the effect on embryogenesis is not only poorly understood but also controversial. It is therefore important to study interspecies differences as results may differ depending on animals used and administration methods.

Key words: Methylxanthines, coffe, tea, cola, prenatal exposure, interspecies studies

Introduction

The group of substances referred to as methylxanthines include caffeine, theophylline, theobromine and aminophylline, which is a compound of theophylline with ethylenediamine. They are mainly absorbed from coffee, tea and cocoa products such as cola beverages and chocolate bars, as well as some medications. Methylxanthines have been demonstrated to exhibit multifunctional physiological effects mediated through molecular processes such as inducing intracellular calcium release via ryanodine receptors, inhibiting phosphodiesterase activity and blocking GABA receptors (Wendler et al. 2009). However, at normal doses they are considered to mainly affect the adenylyl cyclase pathway mediated by adenosine. Of the four characterized adenosine receptors (A1R, A2AR, A2BR and A3R), only A1 – negatively coupled to adenylyl cyclase and A2 – coupled to adenylyl cyclase through Gs protein receptors, have been

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proved to be the main target for methylxanthines (Iglesias et al. 2006).

Gil et al. (1993) reported an inhibitory effect of purinergic receptor antagonists suramin and theobromine on angiogenesis induced in mice by lung cancer cells.

Barcz et al. described an inhibitory influence of theobromine on angiogenic activity and proangiogenic cytokine production (vascular endothelial growth factor, VEGF) of human ovarian cancer cells, and established that the antiangiogenic properties of theobromine are dependent on its interaction with the A2 adenosine receptor (Barcz et al. 1998, Barcz et al. 2000). The inhibitory effect of theobromine on the induction of angiogenesis and VEGF mRNA expression was described by Skopinska-Różewska et al. on the model of v-*raf* transfectants of human urothelial cells HCV-29 (Skopińska-Różewska et al.1998).

VEGF is one of the most important growth factors mediating both ontogenesis and embryonic angiogenesis. In experiments performed on pregnant mice , fed during pregnancy 2 or 6 mg per day of theobromine, Chorostowska-Wynimko et al. found a significant inhibitory effect of this drug on embryo growth and tissue proangiogenic activity. (Chorostowska-Wynimko et al. 2004). These authors also observed a postnatal theobromine effect. The 4-week old progeny of theobromine-fed mothers had significantly shorter limbs and higher spleen mass in comparison to the controls. Moreover, 6-week old progeny of theobromine-fed mothers presented lower a splenocyte response to mitogens (but higher splenocyte graft-versus-host activity) and a higher anti-SRBC antibody response than the progeny of control mice.

Similar results were obtained by Skopiński et al. (2003, 2004) in experiments with pregnant mice fed chocolate. Shortening of limbs was accompanied by lower VEGF content of bones than in control animals and bone mineralization disorder.

Caffeine (1,3,7-trimethylxanthine) is metabolized by demethylation in the liver to paraxanthine, theophylline and theobromine. These metabolites, especially theobromine (also found in cocoa and chocolate) have a high level of toxicity in dogs (Drolet 1984, Strachan 1994, Eteng 1997, Stidworthy 1997). The study of comparative theobromine metabolism in five mammalian species revealed that this compound was most extensively metabolized by rabbits and male mice. Rabbits and dogs metabolized theobromine primarily to 7-methylxantine and 3-methylxantine (Miller et al. 1984). Importantly, caffeine and its metabolites freely cross both the placental and blood-brain barriers due to its hydrophobic properties (Colomina et al. 2002). Additionally, a fetal lack of cytochrome P-450 activity results in a slowed metabolism and therefore accumulation of caffeine (Soellner et al. 2009).

Moreover, the influence of methylxanithines on placental transports of nucleosides has been demonstrated. A study performed on the rat syncytiotrophoblast cell line TR-TBT 18d-1 has indicated the inhibitory effect of caffeine on the placental uptake of uridine and adenosine (Chishu et al. 2008). On the other hand, theophylline has had no effect on nucleoside transport, indicating to the role of the 7-methyl group of caffeine in this process. Tanuma et al. (2003) have indicated increased angiotensin II type (AT2) receptor gene expression in placentas derived from caffeine-administred pregnant rats. Similarly, the expression of the anti-apoptosis regulator B-cell CLL/lymphoma 2 (Bcl-2) gene has also been found to be down-regulated (Nomura et al. 2004). On the other hand, methylxanthine has been demonstrated to exhibit inhibitory effects on the pre-eclamptic-like symptoms in ewes, probably due to interference with the hem metabolism (Talosi et al. 2001).

Nervous system

Considering the fact that there is no blood-brain barrier to methylxanthines and that the adenosinergic system is represented in the brain, caffeine and its metabolites are expected to have an impact on neuronal functions (Black et al. 2008).

The effects of maternal intake of caffeine and theophylline on the adenosine receptors in fetal rat brains have been studied by Leon et al (Leon et al. 2002). In this study A1 receptor down-regulation associated with an increase in A1 mRNA level has been determined by the use of RT-PCR. It has been hypothesized that this antagonist-induced desensitization occurred due to enhanced endogenous adenosine release. In addition, no changes in the A2A receptor have been found. In a follow-up study analysis of the consequences of caffeine administration during gestation was extended to neonatal male and female brains (Lorenzo et al. 2010). More recently, the same group of authors has analyzed the effect of methylxanthine intake during pregnancy on the A1R transduction pathway in fetal rat brains (Leon et al. 2005a). They have detected a significant decrease in hG12 protein in membranes from fetus brains. In contrast(a study conducted by Aden et al. reported no alterations in the adenosinergic system in terms of A1 receptor numbers (Aden et al. 2000).

Also, the influence of in utero caffeine or theophylline exposure on metabotropic glutamate receptor (mGluRs) transduction pathway have been

studied in fetal rat brains (Leon et al. 2005b). The results have indicated that the total number of mGluRs was decreased with no related changes in receptor affinity. Additionally, a down-regulation of other mGluR/PLC pathway components, i.e. mGluR1A, αGq/11 and PLCβ1 has been reported. According to the authors these outcomes can be possibly explained by A1R-mediated inhibition of glutamate release at the presynaptic level.

Moreover, telencephalic vesicle evagination in mouse embryos has been shown to be accelerated by perinatal caffeine exposure (Sahir et al. 2000, 2001). A subsequent study by Sahir et al. has reported that this phenomenon may correlate with an increase in gene expression of the regulatory subunit (RIα) of cAMP-dependent protein kinase (PKA) as well as a decrease in PKA activity (Sahir et al. 2001).

Acetylcholine is considered to be one of the crucial neurotransmitters involved in the neuronal morphogenesis; the release of acetylocholine in the hippocampus and prefrontal cortex is controlled by adenosine (Acquas et al. 2002, da Silva et al. 2008). da Silva et al. (2008) have demonstrated an increase in acetylcholinesterase (AchE) activity with no modifications on mRNA level in the hippocampus of 21-day-old neonate rats. This effect on AChE has been concluded to be caused by phosphorylation mediated by AMPc-dependant protein kinase (PKA).

In addition, laser Doppler flowmetry has indicated that theophylline blocks increases in cerebral blood flow during hypoxia in near-term fetal sheep (Blood et al. 2002). Interestingly, neither cerebral blood flow nor cerebral vascular resistance have been affected by infusion of theophylline in the fetus under normoxic conditions.

Cognitive and behavioral changes

Björklund et al. (2008) have studied mouse offspring born to dams consuming caffeine in drinking water in terms of behavioral changes. They found a positive correlation between perinatal exposure to caffeine and greater locomotor activity per se and in response to cocaine in adult mice. This effect was more notable when the pregnant mice lacked A1 receptors. Hence, the mother's genotype in terms of the adenosine A1 receptor gene (A1R) has been proposed as a key element in determining such long-term alterations. It is also noteworthy that mice heterozygous for the adenosine A1 receptor gene had a motor activity profile paralleling that of caffeine-treated offspring. This effect can be explained by the fact that in both groups signaling via the adenosine A1 receptor is reduced either by decreased gene expression or direct antagonistic influence of caffeine.

Importantly, caffeine has been proved to reduce the motor activity changes in adult animals produced by methylmercury exposure between day 7 of gestation and postnatal day 7 (Björklund et al. 2007).

Moreover, maternal caffeine intake during gestation and lactation leads to reduced hyper-locomotor response to MK-801 in 21-day-old rats (da Silva et al. 2005). MK-801 is the NMDA (N-methyl-D-aspartate) receptor antagonist which is considered to promote hyper-locomotion in rodents. However, this effect may be blunted by chronic treatment with caffeine. In order to test the role of cross-tolerance a washout group subjected to caffeine withdrawal 7 days postnatally was also analyzed. The results showed similarities in motor behaviour between the caffeine-treated group and the washout group, suggesting the permanent character of changes during neurodevelopment.

Furthermore, in adult rats cognitive functions may be adversely affected by chronic prenatal exposure to caffeine (Soellner et al. 2009). Animals submitted to caffeine during gestation present long-term learning and memory deficits tested by novel object recognition and radial arm maze performance.

Respiratory system

The involvement of adenosine A1 receptors in the control of respiration is widely accepted (Herlenius et al. 1997, Herlenius et al. 2002, Gaytan et al. 2006,). They are distributed in many of the areas in the brain associated with the breathing process, such as the ventral respiratory group (VRG) and the pontine respiratory area (Gaytan et al. 2006).

Herlenius et al. have demonstrated that chronic administration of caffeine to pregnant rats during gestation leaded to the increased inhibition from the pontine structures to the neuronal networks in the medulla oblongata in caffeine-treated pups (Herlenius et al. 2002). This effect may be attributed to the methylxanthine-induced increase in activity of pontine noradrenergic neurons. Additionally, no changes in expression of either A1 receptor number or A1 receptor mRNA was detected. The adenosinergic A1 system involvement in respiratory perturbations in newborn rats exposed to caffeine via maternal intake has been investigated by Saadani-Mkki et al. (2004). Based on brainstem-spinal cord preparations, both an overcharge of the respiratory frequency (RF) increase in pontomedullary-spinal cord preparations and an exaggeration of the RF decrease in medullary-spinal cord preparations have been identified. Moreover, in this study the c-fos expression induced by the aden-

osinergic A1 systems acitvation was monitored. The Fos protein is a classical marker of central pathways involved in specific respiratory responses. Hence, this analysis has allowed a positive correlation between the alterations in RF and changed neuronal activity in both the medial parabrachial nucleus and the ventrolateral reticular neurons to be found. In addition, the analysis of consequences of in utero caffeine exposure on respiratory output based on C4 ventral root activity and its correlation with c-fos expression has also been extended to normoxic and hypoxic conditions (Bodineau et al. 2003). Interestingly, in rats ponto-medullary respiratory disturbances caused by in utero caffeine exposure can be prevented by the presence of caffeine in the milk (Bodineau et al. 2006). This seems likely to be possible due to the avoidance of the withdrawal situation in the newborn rats.

Investigations have also been conducted to assess the response to moderate alveolar hypoxia, and both adenosine and benzodiazepine receptors in intact newborn rats exposed to caffeine via the placenta (Picard et al. 2008). The study has found attenuation of both the immediate hyperventilation and the secondary repression during acute alveolar hypoxia in comparison to controls. Furthermore, analysis of Fos expression has suggested decreased efficacy of the 02-sensitive chemoreflex pathway. The use of real-time PCR confirmed that these functional changes were accompanied by increase in A2A receptor and α2 subunit of GABA A receptor on mRNA level in the medulla. The simultaneous alterations of both these receptors may be explained, at least partly, by the fact that A2A recptors are found to modulate breathing processes by the control of GABA release (Mayer et al. 2006, Picard et al. 2008).

Cardiovascular system

Adenosine is a nucleoside distributed in the heart and involved in cardiovascular response (Flood et al. 2002, Xu et al. 2005, Iglesias et al. 2006). Hence, caffeine as a nonselective adenosine antagonist is expected to influence cardiovascular system.

Cardiovascular embryonic function has been assessed by the use of noninvasive high-resolution echocardiography in embryonic mice exposed to caffeine via subcutaneous maternal intake (Momoi et al. 2008). The results have shown transient reduced flows in embryonic carotid artery, dorsal aorta and umbilical artery during the highest caffeine concentration in maternal serum. Both short-term and long-term changes in cardiac development following in utero exposure to caffeine have been tested by Wendler et al. (2009). The study was been performed on 8-10-day embryos and 8-10-week mouse offspring whose dams were intraperitoneally administered a single dose of 20 mg/kg caffeine under normoxic or hypoxic conditions. In embryos outcome of pregnancy in terms of cardiovascular development has indicated a 53% decrease in cardiac ventricular development in hypoxia and 37% in normoxia. Moreover, hypoxia-induced H1F1α protein expression which is regulated by A1R was reduced by 40% upon treatment with caffeine. In offspring a 38% reduction in cardiac function has been confirmed by echocardiography.

Metabotropic glutamate receptors have been proved to be present in the heart and play a role in its physiology (Us et al. 2001, Iglesias et al. 2006). Recently, (Iglesias et al. 2006) have demonstrated down-regulation of mGluRs and a decrease in Gq/11 and PLCβ proteins in fetal rat hearts exposed to caffeine via maternal intake. No changes in mRNA level have been identified, hence these results suggest the involvement of post-transcriptional mechanisms.

Visual system

So far there are only a few reports in the literature on the influence of maternal caffeine intake during pregnancy on the developing visual system. Everekliogluet al. (2004) conducted a histopathologic investigation on lenses isolated from newborn rats whose dam was given caffeine during pregnancy. They found cataractogenic changes pronounced in a dose-dependent manner. No cataract formation was observed in rats whose dams were treated with caffeine at the lowest doses of 25 mg/kg/day. It has been speculated that caffeine-induced caractogenesis was related to the increase in cAMP and its hypoxic-ischaemic necrosis effect on developing crystalline lenses. A negative influence of caffeine on the corneal development has also been noted (Everekliogluet al. 2003). Moreover, the eye opening process may be delayed in rats exposed to caffeine during pregnancy (West et al. 1996).

Combined exposure

Prenatal methylxanthine administration, especially at low and moderate doses, may have little effect when tested as an individual agent. Therefore, the analysis of combined exposure with other components can provide a more realistic view of potential consequences in offspring.

The influence of concurrent exposure to caffeine and restraint stress has been studied in 18-day-old mouse fetuses (Albina et al. 2002). All dams subjected

to caffeine at dose of 120 mg/kg/day and restraint stress died during the study. A significant additive effect has been found in pregnant mice exposed to stress and 60 mg/kg/day of caffeine. This group was characterized by a higher number of late resorptions, postimplantation loss and lower body weight in comparison to the caffeine only treated group. More reduced fetal body weight as well as more frequent cleft palate occurred in the groups concurrently exposed to caffeine and restraint. Interestingly, concurrent exposure had no influence on the number of total implantations, dead fetuses and early resorptions.

In contrast, mouse fetuses exposed to 30, 60 or 120 mg/kg of caffeine and maternal restraint stress on gestational day 9 undervent no developmental toxicity (Colomina et al. 1999).

The effect of co-administration of a single oral dose of 30 mg/kg caffeine, aspirin and a 14-h restraint as a maternal stressor has also been tested (Colomina et al. 2001). Results have shown reduced ossification of ribs and posterior phalanges in 9-day-old mouse fetuses. Reduced ossification of parietal was less frequent in this group in comparison to the controls.

On the other hand, no congenital malformations were noticed in 21-day-old rat fetuses exposed to paracetamol and caffeine during the second week of gestation (Burdan 2003). The paracetamol-caffeine mixture were prepared with a 5:1 proportion and administered at different doses. In this study mean fetal body and placental weight were found to be reduced in a dose-dependent manner.

Black tea brew, which naturally contains caffeine, has been demonstrated to produce no risk in terms of pregnancy outcome in rats (Ratnasooriya et al. 2009). No adverse effects have been noted even upon treatment with high doses corresponding to the consumption of 24 cups/day in humans. This result has been explained by the potential protective influence of other tea components, such as catechins, flavonols, theanine, theaflavins and thearubigins.

Conclusion

In summary, prenatal methylxanthine exposure can induce changes in the developing organism which are persistent even in later phases of life. However, based on results obtained from animal studies, the effect on embryogenesis is not only poorly understood but also controversial. It is therefore important to study interspecies differences as results may differ depending on animals used and administration methods. Future research is needed to fully determine underlying mechanisms and allow extrapolation of the results to humans.

References

Acquas E, Tanda G, Di Chiara G (**2002**) Differential effects of caffeine on dopamine and acetylcholine transmission in brain areas of drug-naive and caffeine-pretreated rats. Neuropsychopharmacology 27: 182-193.

Adén U, Herlenius E, Tang LQ, Fredholm BB (**2000**) Maternal caffeine intake has minor effects on adenosine receptor ontogeny in the rat brain. Pediatr Res 48: 177-183.

Albina ML, Colomina MT, Sanchez DJ, Torrente M, Domingo JL (**2002**) Interactions of caffeine and restraint stress during pregnancy in mice. Exp Biol Med (Maywood) 227: 779-785.

Barcz E, Sommer E, Sokolnicka I, Gawrychowski K, Roszkowska-Purska K, Janik P, Skopińska-Różewska E (**1998**) The influence of theobromine on angiogenic activity and proangiogenic cytokines production of human ovarian cancer cells. Oncol Rep 5: 517-20.

Barcz E, Sommer E, Janik P, Marianowski L, Skopińska-Różewska E (**2000**) Adenosine receptor antagonism causes inhibition of angiogenic activity of human ovarian cancer cells. Oncol Rep 7: 1285-91.

Björklund O, Kahlström J, Salmi P, Ogren SO, Vahter M, Chen JF, Fredholm BB, Daré E (**2007**) The effects of methylmercury on motor activity are sex- and age-dependent, and modulated by genetic deletion of adenosine receptors and caffeine administration. Toxicology 241: 119-133.

Björklund O, Kahlström J, Salmi P, Fredholm BB (**2008**) Perinatal caffeine, acting on maternal adenosine A(1) receptors, causes long-lasting behavioral changes in mouse offspring. PLoS One 3: e3977.

Black AM, Pandya S, Clark D, Armstrong EA, Yager JY (**2008**) Effect of caffeine and morphine on the developing pre-mature brain. Brain Res 1219: 136-142.

Blood AB, Hunter CJ, Power GG (**2002**) The role of adenosine in regulation of cerebral blood flow during hypoxia in the near-term fetal sheep. J Physiol 543: 1015-1023.

Bodineau L, Cayetanot F, Sådani-Makki F, Bach V, Gros F, Lebleu A, Collin T, Frugière A (**2003**) Consequences of in utero caffeine exposure on respiratory output in normoxic and hypoxic conditions and related changes of Fos expression: a study on brainstem-spinal cord preparations isolated from newborn rats. Pediatr Res 53: 266-273.

Bodineau L, Saadani-Makki F, Jullien H, Frugière A (**2006**) Caffeine in the milk prevents respiratory disorders caused by in utero caffeine exposure in rats. Respir Physiol Neurobiol 150: 94-98.

Burdan F (**2003**) Intrauterine growth retardation and lack of teratogenic effects of prenatal exposure to the combination of paracetamol and caffeine in Wistar rats. Reprod Toxicol 17: 51-58.

Chishu T, Sai Y, Nishimura T, Sato K, Kose N, Nakashima E (**2008**) Potential of various drugs to inhibit nucleoside uptake in rat syncytiotrophoblast cell line, TR-TBT 18d-1. Placenta 29: 461-467.

Chorostowska-Wynimko J, Skopińska-Różewska E, Sommer E, Rogala E, Skopiński P, Wojtasik E (**2004**) Multiple effects of theobromine on fetus development and postnatal status of the immune system. Int J Tissue React 26: 53-60.

Colomina MT, Sanchez DJ, Esparza JL, Domingo JL (**1999**) Prenatal effects of caffeine and restraint stress in mice. Proc Soc Exp Biol Med 220: 106-111.

Colomina MT, Albina ML, Sanchez DJ, Domingo JL (**2001**) Interactions in developmental toxicology: combined action of restraint stress, caffeine, and aspirin in pregnant mice. Teratology 63: 144-151.

da Silva RS, Hoffman A, de Souza DO, Lara DR, Bonan CD (**2005**) Maternal caffeine intake impairs MK-801-induced hyperlocomotion in young rats. Eur J Pharmacol 509: 155-159.

da Silva RS, Richetti SK, da Silveira VG, Battastini AM, Bogo MR, Lara DR, Bonan CD (**2008**) Maternal caffeine intake affects acetylcholinesterase in hippocampus of neonate rats. Int J Dev Neurosci 26: 339-343.

Drolet R, Arendt TD, Stowe CM (**1984**) Cacao bean shell poisoning in a dog. J Am Vet Med Assoc 185: 902.

Eteng MU, Eyong EU, Akpanyung EO, Agiang MA, Aremu CY (**1997**) Recent advances in caffeine and theobromine toxicities: a review. Plant Foods Hum Nutr 51: 231-43.

Evereklioglu C, Sari I, Alasehirli B, Guldur E, Cengiz B, Balat Z, Bagci C (**2003**) High dose of caffeine administered to pregnant rats causes histopathological changes in the cornea of newborn pups. Med Sci Monit 9: BR168-173.

Evereklioglu C, Güldür E, Alasehirli B, Cengiz B, Sari I, Pirbudak L (**2004**) Excessive maternal caffeine exposure during pregnancy is cataractogenic for neonatal crystalline lenses in rats: a biomicroscopic and histopathologic study. Acta Ophthalmol Scand 82: 552-556.

Flood AJ, Willems L, Headrick JP (**2002**) Coronary function and adenosine receptor-mediated responses in ischemic-reperfused mouse heart. Cardiovasc Res 55: 161-170.

Gaytan SP, Saadani-Makki F, Bodineau L, Frugière A, Larnicol N, Pásaro R (**2006**) Effect of postnatal exposure to caffeine on the pattern of adenosine A1 receptor distribution in respiration-related nuclei of the rat brainstem. Auton Neurosci 126-127: 339-346.

Gil M, Skopińska-Różewska E, Radomska D, Demkow U, Skurzak H, Rochowska M, Beuth J, Roszkowski K (**1993**) Effect of purinergic receptor antagonists suramin and theobromine on tumor-induced angiogenesis in BALB/c mice. Folia Biol (Praha) 39: 63-8.

Giussani DA, Gardner DS, Cox DT, Fletcher AJ (**2001**) Purinergic contribution to circulatory, metabolic, and adrenergic responses to acute hypoxemia in fetal sheep. Am J Physiol Regul Integr Comp Physiol 280: R678-685.

Herlenius E, Lagercrantz H, Yamamoto Y (**1997**) Adenosine modulates inspiratory neurons and the respiratory pattern in the brainstem of neonatal rats. Pediatr Res 42: 46-53.

Herlenius E, Adén U, Tang LQ, Lagercrantz H (**2002**) Perinatal respiratory control and its modulation by adenosine and caffeine in the rat. Pediatr Res 51: 4-12.

Iglesias I, León D, Ruiz MA, Albasanz JL, Martín M (**2006**) Chronic intake of caffeine during gestation down regulates metabotropic glutamate receptors in maternal and fetal rat heart. Amino Acids 30: 257-266.

León D, Albasanz JL, Ruíz MA, Fernández M, Marttn M (**2002**) Adenosine A1 receptor down-regulation in mothers and fetal brain after caffeine and theophylline treatments to pregnant rats. J Neurochem 82: 625-634.

León D, Albasanz JL, Ruíz MA, Marttn M (**2005a**) Chronic caffeine or theophylline intake during pregnancy inhibits A1 receptor function in the rat brain. Neuroscience 131: 481-489.

León D, Albasanz JL, Ruíz MA, Iglesias I, Martín M (**2005b**) Effect of chronic gestational treatment with caffeine or theophylline on Group I metabotropic glutamate receptors in maternal and fetal brain. J Neurochem 94: 440-451.

Lorenzo AM, León D, Castillo CA, Ruiz MA, Albasanz JL, Martín M (**2010**) Maternal caffeine intake during gestation and lactation down-regulates adenosine A1 receptor in rat brain from mothers and neonates. J Neurosci Res 88: 1252-1261.

Mayer CA, Haxhiu MA, Martin RJ, Wilson CG (**2006**) Adenosine A2A receptors mediate GABAergic inhibition of respiration in immature rats. J Appl Physiol 100: 91-97.

Miller GE, Radulovic LL, DeWit RH, Brabec MJ, Tarka SM, Cornish HH (**1984**) Comparative theobromine metabolism in five mammalian species. Drug Metab Dispos 12: 154-60.

Momoi N, Tinney JP, Liu LJ, Elshershari H, Hoffmann PJ, Ralphe JC, Keller BB, Tobita K (**2008**) Modest maternal caffeine exposure affects developing embryonic cardiovascular function and growth. Am J Physiol Heart Circ Physiol 294: H2248-2256.

Nomura K, Saito S, Ide K, Kamino Y, Sasahara H, Nakamoto T, Abiko Y (**2004**) Caffeine suppresses the expression of the Bcl-2 mRNA in BeWo cell culture and rat placenta. J Nutr Biochem 15: 342-349.

Picard N, Guénin S, Larnicol N, Perrin Y (**2008**) Maternal caffeine ingestion during gestation and lactation influences respiratory adaptation to acute alveolar hypoxia in newborn rats and adenosine A2A and GABA A receptor mRNA transcription. Neuroscience 156: 630-639.

Ratnasooriya WD, Fernando TS (**2009**) Effects of Sri Lankan black tea (Camellia sinensis L.) on pregnancy of rats. Basic Clin Pharmacol Toxicol 105: 361-365.

Saadani-Makki F, Frugiére A, Gros F, Gaytan S, Bodineau L (**2004**) Involvement of adenosinergic A1 systems in the occurrence of respiratory perturbations encountered in newborns following an in utero caffeine exposure. a study on brainstem-spinal cord preparations isolated from newborn rats. Neuroscience 127: 505-518.

Sahir N, Bahi N, Evrard P, Gressens P (**2000**) Caffeine induces in vivo premature appearance of telencephalic vesicles. Brain Res Dev Brain Res 121: 213-217.

Sahir N, Mas C, Bourgeois F, Simonneau M, Evrard P, Gressens P (**2001**) Caffeine-induced telencephalic vesicle evagination in early post-implantation mouse embryos involves cAMP-dependent protein kinase (PKA) inhibition. Cereb Cortex 11: 343-349.

Skopińska-Różewska E, Janik P, Przybyszewska M, Sommer E, Białas-Chromiec B (**1998**) Inhibitory effect of theobromine on induction of angiogenesis and VEGF mRNA expression in v-raf transfectants of human urothelial cells HCV-29. Int J Mol Med 2: 649-52.

Skopiński P, Skopińska-Różewska E, Sommer E, Chorostowska-Wynimko J, Rogala E, Cendrowska I, Chrystowska D, Filewska M, Białas-Chromiec B, Bany J (**2003**) Chocolate feeding of pregnant mice influences length of limbs of their progeny. Pol J Vet Sci 6: 57-9.

Skopinski P, Skopińska-Różewska E, Kamiński A, Dziedzic-Gocławska A, Sommer E, Chorostowska-Wynimko J,

Cendrowska I, Chrystowska D, Sadło J, Siwicki A.K (**2004**) Chocolate feeding of pregnant mice resulted in epigallocatechin related embryonic angiogenesis suppression and bone mineralization disorder. Pol J Vet Sci 7: 131-133.

Stidworthy MF, Bleakley JS, Cheeseman MT, Kelly DF (**1997**) Chocolate poisoning in dogs. Vet Rec 141: 28.

Soellner DE, Grandys T, Nuñez JL (**2009**) Chronic prenatal caffeine exposure impairs novel object recognition and radial arm maze behaviors in adult rats. Behav Brain Res 205: 191-199.

Strachan ER, Bennett A (**1994**) Theobromine poisoning in dogs. Vet Rec 134: 284.

Tálosi G, Németh I, Pintér S (**2001**) Inhibitory effects of methylxanthines on the pre-eclamptic-like symptoms in ewes. Eur J Obstet Gynecol Reprod Biol 99: 25-32.

Tanuma A, Saito S, Ide I, Sasahara H, Yazdani M, Gottschalk S, Nakamoto T, Abiko Y (**2003**) Caffeine enhances the expression of the angiotensin II Type 2 receptor mRNA in BeWo cell culture and in the rat placenta. Placenta 24: 638-647.

Us MH, Ozkan S, Oğuş T, Acar HV, Ege T, Cakir O, Gökben M, Oztürk OY (**2001**) Efficacy of topically applied glutamate-aspartate and pentoxifylline solutions in decreasing myocardial damage during open-heart surgery in rats. J Int Med Res 29: 497-502.

Wendler CC, Busovsky-McNeal M, Ghatpande S, Kalinowski A, Russell KS, Rivkees SA (**2009**) Embryonic caffeine exposure induces adverse effects in adulthood. FASEB J 23: 1272-1278.

West GL, Sobotka TJ, Brodie RE, Beier JM, O'Donnell MW Jr (**1986**) Postnatal neurobehavioral development in rats exposed in utero to caffeine. Neurobehav Toxicol Teratol 8: 29-43.

Xu Z, Park SS, Mueller RA, Bagnell RC, Patterson C, Boysen PG (**2005**) Adenosine produces nitric oxide and prevents mitochondrial oxidant damage in rat cardiomyocytes. Cardiovasc Res 65: 803-812.