Theobromine and the Pharmacology of Cocoa

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Contents

Abstract The effects of theobromine in man are underresearched, possibly owing to the assumption that it is behaviourally inert. Toxicology research in animals may appear to provide alarming results, but these cannot be extrapolated to humans for a number of reasons. Domestic animals and animals used for racing competitions need to be guarded from chocolate and cocoa-containing foods, including foods

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containing cocoa husks. Research ought to include caffeine as a comparative agent, and underlying mechanisms need to be further explored. Of all constituents proposed to play a role in our liking for chocolate, caffeine is the most convincing, though a role for theobromine cannot be ruled out. Most other substances are unlikely to exude a psychopharmacological effect owing to extremely low concentrations or the inability to reach the blood–brain barrier, whilst chocolate craving and addiction need to be explained by means of a culturally determined ambivalence towards chocolate.

Keywords Chocolate · Cocoa · Comparative · Craving · Liking · Myths · Pharmacology · Psychology · Theobromine · Toxicology

1 Background

Chocolate is an excellent example of a dichotomous food commodity. The current scientific and popular media focus on health issues has produced two conflicting health labels for chocolate – antioxidant benefits versus increased risk of weight gain. This is a change from the 1990s, when the focus was on a search for psychoactive constituents of chocolate that would explain not only its appeal, but also its craving-inducing, even its alleged addictive qualities. First phenylethylamine (PEA; Hamilton 1992) and later anandamide (Tytgat et al. 2000) were at the centre of this debate (see Sects. 4.1, 5, respectively).

The only pharmacologically active substance that has generally been ignored in this respect is theobromine, at least in part because of an early and persistent notion that it does not stimulate the central nervous system (CNS) (e.g. "does not show any central activity worth mentioning" – Czok 1974; "ineffective by itself" – Sprugel et al. 1977; "virtually inactive" – Rall 1980, p. 593; "behaviourally inactive" – Snyder et al. 1981; "possesses little pharmacological activity and is almost devoid of effects on the CNS and cardiovascular system" – Gates and Miners 1999; "does not affect the nervous system" – Bonvehi and Coll 2000). This may explain why relatively few studies or reviews on the effects of theobromine have been published, especially in comparison with caffeine. However, some recent findings have created a renewed interest in theobromine. Indeed, although at first glance there appear to be very few relevant publications on the effects of theobromine, the reader will notice that a surprisingly large number of studies and other communications surfaced as work on this chapter progressed.

The main aim of this chapter is to assess the role theobromine plays in the pharmacological activity of chocolate – the main supplier of theobromine to the human diet. In addition, other (potentially) pharmacologically active chocolate constituents will be discussed.

2 Theobromine

2.1 Characteristics

As a purified chemical, theobromine is a white powder, and is mainly produced from cocoa husks as a by-product of chocolate manufacture (The Merck Index 2006), although it can also be synthesised from (3-methyl-)uric acid (The Merck Index 2006; Thorpe 1893, p. 697). It is only very slightly soluble in water (1 g/2,000 ml) and alcohol (1 g/2,220 ml 95%), and only slightly more soluble in boiling water (1 g/150 ml), though it dissolves in dilutions of alkali hydroxides and in mineral acids (The Merck Index 2006; European Pharmacopoeia 2005; IARC 1991).

Theobromine is considered a diuretic, a smooth muscle relaxant, a myocardial stimulant and a vasodilator (Dorland's Illustrated Medical Dictionary 2007). Unlike caffeine, it is a very mild CNS stimulant (Mumford et al. 1994), and it has both antioxidant and pro-oxidant characteristics (Azam et al. 2003).

2.2 Natural Occurrence

Of all structurally related purine alkaloids (methylxanthines), theobromine is the predominant member present in chocolate (Apgar and Tarka 1998). Therefore, chocolate and other cocoa products are the main sources of theobromine in our Western diet. However, it can also be found in small quantities in tea (*Camilla sinensis*; Hicks et al. 1996), guarana (*Paullinia cupana*; Weckerle et al. 2003), mate (*Ilex paraguariensis*; Cardozo et al. 2007) and cola nut (Souci et al. 1981; Burdock et al. 2009), whilst its presence in coffee is negligible at a mere 10% of that in tea (see Table 1 for a general overview of theobromine content in foods).

Note that different tea varieties contain different typical levels of methylxanthines (Hicks et al. 1996). A relatively recently discovered tea variety, *Camellia ptilophylla*, is naturally free of caffeine, but contains high levels of theobromine instead – around 15–18 times the level in of green tea (Yang et al. 2007; He et al. 2009), hence its familiar name "cocoa tea". Likewise, cocoa bean varieties differ in their theobromine content, with Forastero varieties generally containing the highest amounts (Brunetto et al. 2007; Timbie et al. 1978), although some results do not agree with this (Hammerstone et al. 1994). See also Ashihara et al. (2010; Sect. 2.3), and Sect. 2.3.1 below.

2.3 Synthesis, Catabolism and Pharmacokinetics

The brief overview below cannot pretend to represent the complexities of this topic, although I have attempted to cover the most relevant and informative aspects. For a more detailed and in-depth approach, please refer to Ashihara et al. (2010) and Arnaud (2010).

Table 1 Theobromine content of various products. (After Smit and Rogers 2001)

Product	Portion size[a]	Concentration (mg per portion)
Chocolate, dark	50 g	378 (237–519)[b]; 221[c]
Chocolate, milk	50 g	95 (65–160)[b]; 94[c]
Cocoa powder	10 g	189 (146–266)[b]; 203[c]; 260[d] 206 (178–240)[e] 263 (219–284)[f]
Tea (regular, bag)	230 ml	3.1 (1.4–4.4)[b, g]
Coffee (filter/percolated)	7.6 g/200 ml	0.3 (0.3–0.3)[b]
Coffee (instant)	1.6 g/200 ml	0.2 (0.1–0.5)[b]
Cola drinks	Can (330 ml)	ND[h]

ND not detected
[a]MAFF (1988)
[b]MAFF (1998); figures recalculated using comments in Annex C of this reference where appropriate
[c]Craig and Nguyen (1984)
[d]Risner (2008)
[e]Bonvehí and Coll (2000)
[f]De Vries et al. (1981)
[g]This is in accord with values of first brew in Hicks et al. (1996).
[h]Dried kola nut contains 0.05–0.10% theobromine (Souci et al. 1981; see also Duke 1992 in Burdock et al. 2009)

2.3.1 Theobromine Synthesis and Catabolism in *Theobroma cacao*

In the cocoa plant, theobromine accumulates in young leaves, and the concentrations decline as the leaves mature (Koyama et al. 2003). In the cocoa pod, theobromine is synthesised in both the pericarp (fleshy, outer layer) and the cotyledons (seed embryos) of young cocoa fruits, though during the ripening phase, pericarp theobromine concentrations decline sharply, whilst cotyledon (cocoa bean) theobromine concentrations increase. This suggests that the major site of theobromine synthesis is the cocoa bean itself, whilst not excluding a minor role for theobromine migration between pericarp and cocoa bean (Zheng et al. 2004). Whilst theobromine is synthesised from AMP via xanthosine, it is metabolised by demethylation via xanthine, both in the cocoa bean (Zheng et al. 2004; see Ashihara et al. 2008 for a review) as well as in the cocoa leaf (Koyama et al. 2003).

Methylxanthine (including theobromine) concentrations in the cocoa bean are broadly variety-dependent, although publications do not always agree: Brunetto et al. (2007) found cocoa bean theobromine levels varying between 0.7 and 2%, with the highest levels found in the Forastero varieties, whilst the theobromine-to-caffeine ratios varied between 2 and 12, with Criollo, Trinitario and Forastero varieties shown in order of increasing theobromine-to-caffeine ratio. Likewise, Timbie et al. (1978) found cocoa bean theobromine levels of 1.2–3.9%, with the highest average levels found in Forastero, the lowest level in Criollo (which had the highest caffeine content) and increasing theobromine-to-caffeine ratios from Criollo (1.1) through to Forastero (75.1; recalculated from data provided). Hammerstone et al. (1994), however, provided entirely different figures for the same varieties.

Their highest average theobromine content was found in the Criollo varieties (2.3%), with Trinitario, Criollo and Forastero showing increasing theobromine-to-caffeine ratios. Although the analytical procedures are very similar between the publications, minor variations in these procedures may account for some of the differences found. However, the ripening stage at which fruit is picked (Timbie et al. 1978) and possibly also other factors such as growing conditions in terms of soil quality/composition and weather may all affect the methylxanthine content.

Whilst the cocoa beans are being processed (fermentation, roasting, etc.), the theobromine content changes mainly during the fermentation stage. During this stage methylxanthines migrate from the bean into the shell, causing a decrease in cocoa bean theobromine content of around 25% (Timbie et al 1978). Additionally, it is not unreasonable to assume that the microorganisms involved in the fermentation process could further reduce the theobromine content, as is the case with tea (Wang et al. 2008).

2.3.2 Theobromine Uptake, Metabolism and Pharmacokinetics in Man

Following oral administration in man, theobromine absorption from the digestive tract is slow, especially compared with caffeine, with an estimated peak plasma time of 2.5 h (compared with 0.5 h for caffeine) (Mumford et al. 1996). Moreover, theobromine absorption is not complete, at least in some people (less than 90%; Cornish and Christman 1957). Interestingly, the theobromine peak plasma time after chocolate consumption is somewhat faster at 2 h after consumption (Mumford et al. 1996). Although this seems counterintuitive because of plausible increases in the release time from the chocolate food matrix and binding to phenolic compounds (Czok 1974), Mumford et al. (1996) suggested the shorter theobromine peak plasma time following chocolate administration may be caused by stimulating bile production, shown in other studies to improve drug absorption. Note, however, that the same study reported slower caffeine uptake from both chocolate and cola. Despite the explanation provided for the latter (delayed gastric emptying), the plasma concentration curves for both foods are strikingly similar, and suggest a possible sucrose-mediated suppression of the excitatory effects of caffeine (Chauchard et al. 1945). Clearly, more research is needed to uncover the factors relevant to methylxanthine absorption from food.

In humans, methylxanthines are metabolised by demethylation (removal of methyl side groups) by the enzyme cytochrome P450 (CYP). Hence, theobromine (3,7-dimethylxanthine) is broken down to 3-methylxanthine and 7-methylxanthine by CYP. 7-Methylxanthine is then further metabolised into 7-methyluric acid by xanthine oxidase (this is not the case for 3-methylxanthine), whilst metabolism of theobromine into 3,7-dimethyluric acid and 3,7-diaminouracil is less well understood, although this is at least in part CYP-mediated (Gates and Miners 1999).

Note that theobromine does not metabolise into other dimethylxanthines (i.e. theophylline or paraxanthine), nor does it “upgrade” to the trimethylxanthine

caffeine (Mumford et al. 1996), although the latter does happen in young leaves of the *Theobroma cacao* plant (Koyama et al. 2003). However, humans are exposed to theobromine though demethylation of caffeine, in addition to the ingestion of theobromine.

The clearance rate for acutely administered theobromine is around 1.2 ml/min/kg, around half of that of caffeine (Lelo et al. 1986), whereas after 4 days of chronic administration, Miners et al. (1982) found a clearance rate of 0.75 ml/min/kg. Likewise, Drouillard et al. (1978) found acute theobromine clearance rates of 0.94 ml/min/kg (1.47 after a 2-week methylxanthine abstinence), reduced to 0.81 after 5 days of chronic administration (figures calculated from published data), suggesting that the chronic exposure-related reduction in theobromine clearance is reversed after dietary theobromine abstinence (Drouillard et al. 1978). Note that interindividual differences in theobromine clearance rates may be substantial, as is the case for caffeine (Lelo et al. 1986 measured a 1.2 $\pm$ 0.4 ml/min/kg theobromine clearance rate; Balogh et al. 1992 measured 79% interindividual variance in caffeine clearance rates). Moreover, tobacco smokers have a substantially increased theobromine clearance compared with non-smokers (Miners et al. 1985).

2.4 Mechanism of Action

Although various effects of caffeine have in the past been attributed to the release of intracellular calcium and inhibition of cyclic nucleotide phosphodiesterases, ordinary human consumption of dietary methylxanthines would be insufficient to reach the levels needed for these processes to be activated (Fredholm et al. 1999). The main mechanism of action for methylxanthines has long been established as an inhibition of adenosine receptors (Snyder et al 1981; see Fredholm et al. 1999 for an extensive review). A range of secondary effects of adenosine antagonism may explain the variety of effects of methylxanthines on the human system in more detail. The interaction of adenosine A_{2A} receptors with dopamine D_2 receptors (Fredholm et al. 1999) is one such example. Interestingly, theobromine shows a much lower affinity for adenosine receptors than caffeine (Daly et al. 1983; Fredholm and Lindström 1999), which may explain why it is generally regarded as behaviourally inert. However, caffeine and theobromine show differential affinities for different adenosine receptor subtypes. Daly et al. (1983) found that theobromine is 2–3 times less active than caffeine as an adenosine A_1 receptor antagonist, but at least 10 times less active than caffeine as an A_2 receptor antagonist. Fredholm and Lindström (1999) gave similar values, but with a clear difference in caffeine-to-theobromine affinity ratios for stratium compared with cortex A_1 receptor antagonism (theobromine was found to be 4.7 and 11.8 times less active than caffeine, respectively). Nevertheless, the authors suggested caffeine and theobromine are non-selective receptor antagonists.

Interestingly, the much higher presence of theobromine in chocolate compared with that of caffeine (theobromine-to-caffeine ratio average 10; milk chocolate

11.3; dark chocolate 14.0; cocoa powder 9.0; recalculated from Tables 1 and 2 in Smit and Rogers 2001), clearly do not make up for the lower average adenosine receptor affinity of caffeine compared with that of theobromine (again, of around a factor 10 in Fredholm and Lindström 1999, akin to the difference in locomotor stimulation threshold between caffeine and theobromine reported by Snyder et al. 1981). Moreover, because A_1 receptors determine the effects of caffeine on fluid intake (Rieg et al. 2007), whilst the A_{2A} receptors play a role in the desire for caffeine (El Yacoubi et al. 2005), the differential affinities for different receptor types provide a possible explanation for the observation that caffeine and theobromine exert different effects. Note that additionally, the caffeine dimethylxanthine metabolites paraxanthine and theophylline have adenosine receptor affinities even stronger than caffeine (Daly et al. 1983; Fredholm and Lindström 1999), thereby explaining part of the effects of caffeine, whilst theobromine, also a dimethylxanthine, does not have such metabolites. Moreover, the reduced and delayed uptake of theobromine compared with that of caffeine may further diminish the in vivo effect of theobromine as an adenosine receptor antagonist in terms of its central and peripheral effects.

2.5 Effects in Animals

The effects of theobromine in animals as reported in the scientific literature can broadly be categorised into three groups: (1) toxicology studies; (2) case studies or reports of theobromine poisoning; (3) pharmacology studies; and (4) behavioural studies. Additionally, concern regarding the use of theobromine as a doping agent in equine and related sports has also penetrated the scientific literature.

Dietary theobromine intake in animals originates from two sources: (1) domestic chocolate and chocolate- or cocoa-containing foods as consumed by humans; (2) animal feed containing cocoa shell. The use of cocoa shell in animal has seen a drastic increase since the discovery that (1) it contains high levels of vitamin D, (2) its addition to the cattle's winter diet raised the vitamin D level to that which it typically is during the summer months, and (3) milk fat content was also raised when using this feed (Knapp and Coward 1934; Kon and Henry 1935; Golding and Burr 1937 in Dowden 1938). It is likely that experience from the use of this feed taught the equine sports that it was beneficial to animal performance, though this is not clear from the literature. Note that McDonald et al. (2002; p. 596) mentioned another feed derived from the cocoa bean, that is "extracted cocoa bean meal", which also contains theobromine and which the authors therefore also did not recommend being fed to racing horses. Moreover, the European Food Safety Authority has mentioned cocoa bean meal, cocoa husk meal, cocoa germs, cocoa bean shells and discarded chocolate confectionery as sources for animal feed in Europe (EFSA 2008).

2.5.1 Toxicology Studies

Toxicology studies mainly concern teratology and male reproductive toxicology, presumably following a study by Friedman et al. (1979), which reported testicular atrophy in nearly all rats fed caffeine or theobromine at a dietary concentration of 0.5% for over 14 weeks, although the detrimental effects in the caffeine condition were greater. However, Gans (1984) reported the reverse, that is, testicular atrophy and spermatogenic cell destruction following feeding with theobromine were much greater than they were following feeding with caffeine. Though the latter study used a dietary concentration of 0.8% theobromine with an exposure time of 7 weeks, subsequent studies switched to daily doses of 25–500 mg/kg body weight (Wang et al. 1992), and have shown similar effects for a shorter test duration, even after 2 weeks (Funabashi et al. 2000). Lower toxicity has been shown for cocoa powder containing the same amount of theobromine (Wang and Waller 1994). Additionally, Tarka et al. (1981) showed that when rats were fed chow containing 0.6 and 0.8% theobromine for 7 weeks, testicular weight decreased significantly compared with feeding with 0 and 0.2% theobromine. Moreover, they showed this effect was irreversible as measured during the subsequent 7 weeks. Although the underlying mechanism is unclear, its effects are seen also in terms of degeneration and necrosis in spermatogenic cells (Gans 1982; Wang and Waller 1994). Similarly to theobromine, cocoa powder at 5% of the diet showed testicular atrophy and decreased spermatogenesis (Tarka et al. 1991). The effects of theobromine on the male reproduction system described above have been validated in several other publications (Weinberger et al. 1978; Soffietti et al. 1989; Tarka et al. 1979). Note that similar atrophy effects have been observed for the thymus gland in rats (Tarka et al. 1979), appearing sooner than testicular damage (Gans 1982), and with theobromine producing higher decreases in thymus weight than caffeine (Gans 1984), though these effects were not found in dogs (Gans et al. 1980). Because this gland "plays an important role in cellular immunity by generating circulating T lymphocytes" (Nishino et al. 2006), the effects of theobromine reported on this gland may suggest an increase in overall immune response suppression.

The toxic effects of theobromine also include growth reduction and weight loss, possibly achieved through loss of appetite and food intake (Tarka et al. 1979).

Theobromine doses as low as 6 mg/day in the diet of mother mice reduces embryo weight and embryo tissue angiogenic activity (i.e. the rate at which new blood vessels are formed in growing tissue), and reduces neonatal relative limb size and spleen weight, suggesting that this is caused by a theobromine-induced reduction in the formation of new blood vessels in embryos (Chorostowska-Wynimko et al. 2004). The same research group showed a similar effect of chocolate (Skopinski et al. 2004) but attributed this to its epigallocatechin content owing to the correlations found between effect size and epigallocatechin content. Although this appears strange as the theobromine concentrations would have produced the same conclusion for theobromine and confirmed the results of their other publication of the same year, yet another study confirmed the link between dietary cocoa flavanol dose and embryonic (and tumour) angiogenesis (Wasiutynski et al. 2005).

Further studies will need to point out differential roles or mechanisms for these effects of theobromine and cocoa polyphenols, respectively, and evidence for similar effects in man ought to be sought. Nevertheless, Tarka et al. (1986a, b) pointed out that at much lower doses (25–200 mg/kg body weight/day), only their highest doses showed teratogenic effects (a delay in osteogenesis in rats; maternal toxicity/mortality, fetal malformations and osteogenic delays in rats), whilst the theobromine intake in these doses in rats and rabbits would be equivalent to an unrealistic human consumption of 7.5–10 lb (3.4–4.5 kg) milk chocolate/day, possibly explaining why no human teratogenic effects of theobromine have been reported. Alternatively, a 5% cocoa powder as used by Tarka et al. (1991) would not be impossible to implement in the human diet, though the effects of this on the male (and the female) reproduction system are unknown.

Interestingly, and in line with the findings reported above, angiogenesis in tumour growth is also inhibited by theobromine, an example of how a toxic effect can have a positive outcome (eBarcz et al. 1998; Gil et al. 1993). This effect is mediated through inhibition of adenosine receptors (Barcz et al. 2000) present in the carcinoma itself (Ryzhov et al. 2008) and their role in carcinoma hypoxia (Ryzhov et al. 2007), which would explain why similar effects are found with caffeine (Merighi et al. 2007). Conversely, theobromine intake has been associated with the prevalence of prostate (Slattery and West 1993) and testicular (Giannandrea 2009) cancer, although these associations were inconsistent over several decades Giannandrea (2009), and have not been tested further. Nevertheless, theobromine can reduce copper, thereby generating oxygen radicals (Shamsi and Hadi 1995 in Schmid et al. 2007). Moreover, because caffeine can impair DNA double strand repair (Sarkaria et al. 1999), it is possible this may also apply to theobromine, lending theobromine, as is the case for caffeine (Azam et al. 2003), both pro- and anticarcinogenic properties. Investigating the effects of cocoa powder, Tarka et al. (1991) found no evidence of a carcinogenic effect. However, the phenolic content of cocoa is likely to counteract any carcinogenic activity of other cocoa constituents (Lee et al. 2006; Jourdain et al. 2006).

Note that the toxic effects of theobromine may depend on other dietary constituents (e.g. protein content) and species-specific tolerance levels. Therefore, the marked differences in theobromine's toxic effects observed between animal species may make extrapolations to the human system very complex (Tarka et al. 1979), if not impossible.

2.5.2 Case Studies of Animal Poisoning

Many cases of animal poisoning reportedly result from the consumption of chocolate. Dogs, unlike cats, find chocolate a most palatable food, and are therefore most vulnerable to chocolate poisoning, especially when kept indoors. Strachan and Bennett (1994) reported acute cardiac arrest in a dog on the morning after the consumption of cocoa powder on the evening before, with an estimated theobromine exposure of 80 mg/kg body weight. Stidworthy et al. (1997), however, reported

similar symptoms in two dogs who died within 1 h after an estimated consumption of 20–30 g dark chocolate each (using Table 1 and the reported average animal weight of 24 kg, this equates to an estimated theobromine exposure of 8 mg/kg), whilst two similar animals fed the same appeared unaffected. Interestingly, Gans et al. (1980) showed that acute doses of 200 mg/kg and less were not lethal. Other cases of dog poisoning have been reported following the consumption of garden mulch made of chocolate beans and shells, although these animals were successfully treated and recovered within 5 days (Hovda and Kingston 1994). The symptoms are varied, but include vomiting, restlessness, diarrhoea, haematuria (blood in urine), tachycardia (rapid heart beat) and hyperpnoea (deep breaths due to hypoxia) (Hovda and Kingston 1994), shivering and convulsions (Strachan and Bennett 1994), and panting, restlessness and muscle tremors (Gans et al. 1980).

However, deaths following the consumption of chocolate have also been found in wildlife. Reportedly, parrots (Gartrell and Reid 2007), foxes and badgers (Jansson et al. 2001), and undoubtedly more animal species, have been the victim of the consumption of chocolate left unattended.

Even the consumption of cocoa products as an ingredient in cattle feed or other animal feed (i.e. cocoa meal, cocoa husks or chocolate waste from the food or catering industry) can lead to livestock poisoning, even death (e.g. poultry – Black and Barron 1943; calves – Curtis and Griffiths 1972; ducks – Gunning (1950); fowl, ducks and horses – see Blakemore and Shearer 1943 for a review of several early cases).

The toxicity of chocolate to animals has inspired research into coyote pest control in the USA, resulting in an optimal mortality caffeine-to-theobromine ratio of 1:5 (Johnston 2005), not dissimilar to that of chocolate and other cocoa products, reconfirming the danger of this food in domestic animals. Note, however, that the latter publication reiterated the importance of the *combination* of caffeine and theobromine in the effects found, suggesting a focus on theobromine alone as the active toxicant it is not justified when toxic effects or death are caused by the consumption of chocolate.

2.5.3 Equine Sports and Theobromine Doping

In equine sports, caffeine and theobromine are considered doping agents owing to their stimulant effects. Hence, horse urine should not contain any caffeine (exposure detection level set at 0.1 μg/ml), whilst theobromine levels should not exceed 2 μg/ml (IFHA 2007). Although this appears to be a fairly generous level for a doping substance, this can be easily exceeded by feeding a horse 20 chocolate-coated peanuts per day (equivalent to 1.5 such peanuts per day for a human being on a weight basis), and could therefore be interpreted as extremely conservative (Budhraja et al. 2007). Logically, the use of by-products from the cocoa industry in horse feed also increases urine theobromine levels (Haywood et al. 1990), again increasing the risk of doping detection. Upon theobromine exposure, Delbeke and Debackere (1991) recommend a 2-day washout period to ensure urinary

theobromine levels are below the legal threshold, although for other methylxanthine-containing foods, e.g., guarana, this may be insufficient: Salvadori et al. (1994) identified theobromine in horse urine up to 318 h (13 days) after guarana administration. Moreover, like many other drugs, toxins and trace elements and/or their metabolites, theobromine can also be detected in equine hair as a means for assessing drug history (Dunnett and Lees 2003). Whilst methylxanthine doping is also an issue in greyhound racing (Wells et al. 1988; Loeffler et al. 2000) it would be interesting to see if the relevant sports organisations will follow the example of the World Anti-Doping Agency of moving caffeine from the "Prohibited List" to the "Monitoring Program" for detecting patterns of misuse rather than imposing a ban. The reasons for this change include (1) the presence of a great interperson variability in caffeine metabolism, (2) the notion that above the traditionally used 12 μg/ml threshold level, caffeine has a detrimental effect on performance, but also (3) that lowering the detection threshold increases the risk of being penalised for consuming caffeine through everyday food and drink (WADA 2008). It is likely that some, if not all, of these arguments are applicable to dogs and horses, where chocolate treats and potential contamination of feed with cacao may impose more problems than the benefits for both racing organisations and competitors.

Much like horse racing in Western countries, camel racing is as important a sport in, for example, the United Arab Emirates, where methylxanthines are assessed in camel urine using a zero-tolerance approach in doping control (Wasfi et al. 2000).

2.5.4 Pharmacology Studies

Unlike toxicology studies, only a few studies have investigated theobromine metabolism in animals, one of which recorded this in detail in rats (Bonati et al. 1984), and did not find a clear difference between acute and chronic administration on the pharmacokinetics, though the absorption rates declined with increased theobromine doses. Shively and Tarka (1983) found that theobromine metabolism was slower in rats than in humans, whilst in rats it was not affected by pregnancy status. Moreover, in a study comparing five mammalian species (rats, mice, hamsters, rabbits and dogs), Miller et al. (1984) found that theobromine was most extensively metabolised in male mice and rabbits, and that theobromine metabolism shows only quantitative differences between species and sexes.

2.5.5 Behavioural Studies

Kuribara and Tadokoro (1992) reported that the mean 3-h post-treatment ambulatory activity in mice was increased after oral doses of both 10 mg/kg theobromine and 1 g/kg cocoa powder, whilst response rates were increased in the shuttle avoidance task at 3 mg/kg theobromine. However, the performance in the avoidance tasks was disrupted at 100 mg/kg theobromine or higher (Kuribara and

Tadokoro 1992). Similar results were reported by the same group in a different paper (Kuribara et al. 1992), where only the 10 mg/kg theobromine dose increased the avoidance rate in mice, and where at the 1,000 mg/kg dose, half of the mice died within a few hours. Because the measurements were taken over a 3-h period, this may explain why Snyder et al. (1981) found no effect on locomotor activity in mice at 5–100 μmol/kg (1–18 mg/kg) during their 1-h post-treatment observation. He et al. (2009), however, found no effects of 30 mg/kg theobromine or 200 mg/kg cocoa tea (*Camellia ptilophylla*; see Sect. 2.2) on ambulatory behaviour in mice during a 2h post-treatment observation period. Instead, they reported that only in combination with caffeine (as chemicals or as green tea) was a synergistic effect found compared with caffeine alone. Although the caffeine dose (10 mg/kg) at which the synergistic effects with theobromine were shown would have been unusually high in humans and surely not relevant to chocolate consumption, this study provides important evidence for furthering our understanding of the behavioural effects of the methylxanthines in chocolate. Conversely, Heim et al. (1971) and Sprugel et al. (1977) found that locomotive activity, oxygen consumption and brain cyclic GMP and cyclic AMP levels in white mice were affected by caffeine, but that this effect was prevented by theobromine, whilst theobromine itself did not affect these measures (Heim et al. 1971; Sprugel et al. 1977). Only 2–3 h after treatment did effects of theobromine alone occur (Heim et al. 1971). Moreover, after caffeine versus saline discriminative stimulus training in male Sprague-Dawley rats, several methylxanthines, but not theobromine, generalised to the caffeine cue at most doses tested (10–75 mg/kg for theobromine; Carney et al. 1985). These findings suggest that, at least in mice, the theobromine concentrations in chocolate may have a behavioural consequence, that this consequence is of an interactive nature with other methylxanthines, and that behavioural effects of theobromine may be delayed compared with those of caffeine.

Only a few other animal species have been the subject of investigations regarding behavioural effects of theobromine. After previously having identified some purines and other potentially behaviourally active substances from hornet queens, Ishay and Paniry (1979) investigated the effects of the main methylxanthines on hornet behaviour. They found that unlike the effects of purine and hypoxanthine, the effects of caffeine, theobromine and theophylline included nervousness, shaky movement and unsteady gait, reduced physical contact and positive geotropism, with no marked differences between the methylxanthines.

2.6 Effects in Man

Although theobromine is the most predominant methylxanthine present in chocolate, research into the effects of theobromine in man is relatively scarce compared with that into the effects of caffeine, and compared with research in animals. This section aims to present the research on theobromine in man to date.

2.6.1 Psychopharmacological Effects

Several inappropriately substantiated popular claims about the psychopharmacological activity of chocolate constituents (e.g. PEA, see later) resulted in the investigation of the ecological potential of a range of such substances (Smit and Rogers 2001). It was concluded that caffeine and theobromine were the only likely substances to play a role in the psychopharmacological activity of chocolate. This idea was confirmed when the same authors (Smit et al. 2004) showed that the combination of caffeine (19 mg) and theobromine (250 mg) contained in a 2-oz bar (approximately 50 g) of dark chocolate has significant effects on energetic arousal, reaction time and information processing. Subsequent work reported that the same combination of methylxanthines increased the liking for the flavour of a 'novel' drink when combined with the (encapsulated) active substances compared with an encapsulated 'placebo' (Smit and Blackburn 2005). These results show a role for chocolate methylxanthines in our liking for chocolate. Additionally, they provide a very clear explanation for why we prefer milk chocolate over white chocolate, and why dark chocolate is an easily acquired taste. However, a study comparing the individual effects of caffeine and theobromine with the effect of their combination (as used in Smit et al. 2004) using identical, ecologically valid amounts has not been performed to date. Such a study would clarify whether the effects found are either solely or partly attributable to caffeine, and whether caffeine and theobromine provide an additive or synergistic effect.

Only a very few early publications have reported individual and combined effects of caffeine and theobromine. Dorfman and Jarvik (1970) gave volunteers 300 mg caffeine and/or 300 mg theobromine before the volunteers retired for the evening. Those in the caffeine and caffeine + theobromine condition showed a longer sleep latency and lower sleep quality than those in the theobromine condition. Additional data confirmed that sleep latency increases were related to caffeine dose and not to theobromine. Finally, they did not find any interactive effects of the two methylxanthines.

In a study of a more exploratory nature, Mumford et al. (1994) provided some valuable insights into the comparative effects of caffeine and theobromine on mood and cognition by investigating their subjective effects. Despite the small sample size ($N = 7$), and the use of relatively high doses of methylxanthines [the doses used were the lowest discriminable caffeine dose in the least sensitive volunteer (178 mg) and the highest tolerated dose of theobromine by the most sensitive volunteers (560 mg)], this study presented some very interesting and important findings. First of all, it shows how theobromine possesses caffeine-like qualities by means of the subjective effect descriptions of the most theobromine sensitive participant: "Energy", "Motivation to work", "Alert", "Sleepy" (decreased), whilst these effects were emphasised by an additional effect on the measure "Magnitude of drug effect". Second, the discrimination threshold phase of the study showed a wide range of reliable discrimination thresholds amongst the volunteers, although this was not further investigated. This study only provided limited information with

regard to the role of the individual methylxanthines in the psychopharmacological effects of chocolate, although clearly a role for the effects of theobromine cannot be ruled out, and may depend on the individual's sensitivity to these effects.

Further evidence for caffeine-like effects of theobromine, albeit anecdotal, was provided by Ott (1985; pp. 79–80), who replaced his dietary caffeine intake with a daily dose of 600 mg theobromine (200 mg in the morning, afternoon and evening) for 7 days. Upon acute theobromine deprivation, the author described how he "developed a tension headache, muscle tension in his shoulders and neck, and became extremely lethargic" within 16 h. These symptoms were reversed within 60 min of the consumption of another 200-mg dose of theobromine, suggesting that the symptoms were that of theobromine withdrawal. Because this one-man experiment was not performed according to double-blind conventions, Ott advocated that the scientific community carry out a proper study looking into these effects.

In summary, theobromine produces only very minor subjective effects compared with caffeine. In sensitive individuals these effects may be more marked, but can also be detrimental in the form of headaches (Mumford et al. 1994), as can caffeine. However, anecdotal evidence suggests that theobromine behaves like caffeine by means of its capability of producing withdrawal and providing subsequent withdrawal-reversal effects. Unfortunately, no data on the effects of theobromine on mood and cognition in humans other than those presented above have been reported, confirming that this area is seriously underresearched. Although the psychopharmacological effects of theobromine may be smaller than those of caffeine, they have been reported. Taking into account habitual caffeine and theobromine intake, and discriminable and/or tolerable doses, these may help to provide a more sensitive method for uncovering clearer effects of theobromine on mood and mental performance.

2.6.2 Physiological Effects

Cardiovascular

Theobromine is generally regarded both as a bronchodilator and as a vasodilator (Reynolds 1993) and may therefore have an effect on the heart. Indeed, Czok (1974) claimed theobromine provides an effect of medium strength on the heart in general, an effect less strong than the related theophylline, but stronger than caffeine. However, no more precise explanation than that was provided, nor were any citations listed. Effects of theobromine on the heart were confirmed by anecdotal evidence reported in Ott (1985, p. 82), where the author described experiencing cardiac-stimulating effects of an oral dose of 200 mg theobromine within 15 min of administration. Interestingly, theobromine has also been prescribed for relief from pain caused by angina pectoris in some patients, presumably by means of its vasodilating effects (Dock 1926). Although Baron et al. (1999) did not find any cardiac or haemodynamic effects of theobromine, it is possible that the cocoa polyphenols in their chocolate may have obscured any theobromine-related effects.

Note that other studies have also investigated haemodynamic effects of chocolate, but attributed these effects to cocoa polyphenols (Taubert et al. 2003; Grassi et al. 2005) whilst not taking into account the potentially confounding effects of theobromine, although Kelly (2005) argued for this to be addressed.

Geraets et al. (2006) found strong inhibitory effects of theobromine on the activity of the nuclear enzyme poly(ADP-ribose) polymerase-1 (PARP-1), which is implied in acute and chronic inflammatory diseases such as stroke, ischaemia–perfusion and diabetes, and implied in chronic obstructive pulmonary disease. For this reason, they emphasised that methylxanthines (including theobromine) with higher PARP-1 inhibition rates are potentially helpful dietary agents in the treatment of vascular dysfunction and inflammation.

On balance, very few studies have been published investigating cardiovascular effects of theobromine, though some limited evidence suggests that theobromine exerts cardiovascular effects by means of vasodilation and cardiac stimulation. Because the effects of caffeine on cardiovascular functions are expressed though noradrenalin release from sympathetic nerves acting on α_2-adrenergic receptors, with a possible, but much less important role for adenosine (A_1) receptor antagonism (Fredholm et al. 1999), similar effects of theobromine can be expected, although possibly of lower magnitude. Finally, further research is needed to validate the hypothesis that theobromine can be used for the prevention and treatment of vascular dysfunction and inflammation.

Respiratory

Theobromine improves bronchodilation in asthma patients (Simons et al. 1985), although this effect is stronger with theophylline and caffeine (Becker et al. 1984). However, note that the order of bronchodilation efficacy for these three methylxanthines is different in Apgar and Tarka 1999, who listed theobromine as stronger than caffeine for this effect. Presumably owing to its superior diffusion in bronchial tissue (van Zyl et al. 2008), theophylline (1,3-dimethylxanthine) is still used as a medication for asthma patients but can have serious side effects (Barnes and Pauwels 1994; El-Bitar and Boustany 2009), whilst caffeine and theobromine are not in use as such. Nevertheless, caffeine does improve lung function (Bara and Barley 2001), as is also supported by epidemiological evidence (Pagano et al. 1988), suggesting that asthma and bronchitis patients may be self-dosing on caffeine to relieve symptoms, even if this is subliminally achieved by means of positive reinforcement. Because theobromine *also* improves lung function (10 mg/kg; Simons et al. 1985), there may be a similar role for the consumption of chocolate in the relief of asthmatic symptoms. Indeed, I have anecdotal evidence (from a personal acquaintance whose partner is suffering from asthma) of a clear association between periods of heightened asthmatic symptoms and a marked increase in consumption of both chocolate and cola drinks. Interestingly, it was not the patient herself, but was her partner who became aware of this association. Note that whilst the tobacco industry claims to add cocoa powder to cigarettes as a

flavouring agent, it may also conveniently serve to enhance the uptake of nicotine (and thereby increase the addictive property of tobacco) through the bronchodilating properties of theobromine (Rambali et al. 2002), as well as possibly suppressing smoke-induced cough reflex (see below).

A complementary beneficial effect of theobromine on the airways relates to theobromine's more recently identified cough reflex suppressant ("antitussive") properties through suppression of vagus nerve activity. This effect was shown in response to both inhalation of a citric acid aerosol in guinea pigs and to inhalation of a capsaicin aerosol in humans. Interestingly, although there was no clear difference between theobromine and codeine in suppressing citric acid induced cough in guinea pigs, the suppressant effects of theobromine on cough induced by a capsaicin aerosol in human volunteers were greater than those of codeine. Moreover, and unlike codeine, theobromine was free from side effects (Usmani et al. 2005), an important notion in the context of a strong need for antitussives without side-effects (Chung and Chang 2002). This could lend theobromine a direct medical application in the reduction of cough, as cough is a common symptom in cancer (Walsh et al. 2000), and usually responds well to one or more medications (Table 1 in Estfan and Walsh 2008). This application could be extended to chocolate, where a corresponding portion of dark chocolate could reduce the cost of conventional medicine where proven effective. Hence, dark chocolate is currently being investigated as an alternative to medicine for its potential to reduce cough in cancer patients for whom cough is a troubling symptom (Halfdanarson and Jatoi 2007).

Though seemingly related, not in the least by the effect theobromine has on either, the regulation of smooth muscle relaxation and that of cough suppression are different. Smooth muscle relaxation is regulated by β_2-adrenergic receptors (whereby β_2-adrenergic receptor agonists acutely improve bronchodilation, although chronic exposure can have detrimental effects on the control of asthma; Lipworth and Williamson 2009), and by adenosine receptors (Brown et al. 2008). Although methylxanthines have bronchodilating effects and act as adenosine receptor antagonists, this may not be the main mode of action for theobromine as a bronchodilator or smooth muscle relaxant. Indeed, Lunell et al. (1983) reported strong bronchodilator effects of a xanthine derivative without any CNS effects. However, both A_1 and A_{2B} receptors have been implied in the pathogenesis of asthma, and although roles for A_{2A} and A_3 receptors are likely, they are still unclear (Brown et al. 2008).

The cough reflex is triggered by three different kinds of sensory nerve receptors in the respiratory tract, whose signals are relayed via the vagus nerve and the brainstem to the "cough centre" or "central cough generator", where the physical cough response is coordinated (Chung and Pavord 2008). Although current antitussives are mainly opiates and opiate derivatives acting on the central cough pathway, their side effects call for the development of other substances that achieve the same goal through different mechanisms. Therefore, new and proposed antitussives acting centrally may target sigma or GABA receptors, or act through other mechanisms yet to be identified (Reynolds et al. 2004). Alternatively, they may act peripherally by directly targeting neuronal pathways, for example, ion channels, nerve fibres and relevant receptor sites (Chung and Chang 2002; Reynolds et al. 2004). Moreover,

guinea pig sensory nerve activity and human sensory nerve activity in the airways are inhibited by activating cannabinoid CB_2 receptors (Patel et al. 2003; Belvisi et al. 2008). Although Usmani et al. (2005) showed that theobromine also inhibits guinea pig vagus nerve activity, its modus operandi has not been established. Nevertheless, the authors suggested that theobromine is likely to exert its effect through suppression of phosphodiesterase activity and by inhibiting bronchoconstricting adenosine A_1 receptors, though alternative modes of action (e.g. activation of Ca^{2+}-activated K^+ channels) cannot be ruled out (Usmani et al. 2005).

Concluding, both the antitussive and the bronchodilating effects of theobromine are at least in part related to the adenosine receptor antagonistic properties of theobromine as part of the methylxanthine family, and it could be that different effects are expressed through different adenosine receptor subtypes and though other receptors, such as β_2-adrenergic receptors. The exact pathways for the bronchodilating and antitussive effects of theobromine are unclear, and whilst other pathways may be involved, further investigation is clearly needed to clarify this topic.

Renal

Because adenosine plays an important role in regulating blood flow, it also plays an important role in renal haemodynamics, affecting renal blood flow and glomerular filtration rates. The renal vascular system, however, unlike the main vascular system, is regulated by adenosine A_1 receptors in addition to A_2 receptors (see Hansen and Schnermann 2003; Vallon et al. 2006 for reviews of the role of adenosine in the kidney). The finding that A_1 receptors also determine the effects of caffeine on fluid intake (Rieg et al. 2007) may be related to this. Because theobromine has a lower overall adenosine receptor affinity than caffeine and theophylline, though all three methylxanthines are non-selective adenosine antagonists (Fredholm and Lindström 1999), a small, but possibly insignificant, diuretic effect of theobromine would be predicted not to be functionally different from the other methylxanthines. Indeed, despite a previous and unjustified claim that theobromine has a stronger effect on the kidney than caffeine (Czok 1974), Dorfman and Jarvik (1970) and Massey and Whiting (1993) reported that unlike caffeine, oral administration of 300 mg theobromine did not increase urinary calcium or sodium excretion, although Dorfman and Jarvik (1970) found no change in the overnight urine volume following oral administration of 300 mg caffeine or 300 mg theobromine compared with ‘no drug’.

Dental

The consumption of chocolate, as a sugar-containing confectionery, is inevitably associated with dental caries (i.e. chocolate is seen as a cariogenic food). However, both theobromine (added to the diet in hamsters – Strålfors 1967; applied to human teeth in vitro – Sadeghpour 2007), and cocoa (reviewed in Naylor 1984) reportedly

inhibit dental caries. Sadeghpour (2007) found that regular exposure to theobromine increased the enamel surface microhardness compared with sodium fluoride, and helped surface recrystallisation. Kashket et al. (1985) found that defatted cocoa inhibits dental plaque formation, as did cocoa extracts (Srikanth et al. 2008) and cocoa polyphenol extracts (Percival et al. 2006). Whilst the preparations used in the work reported in the latter publication may or may not have been free of methylxanthines, the authors did not make any reference to this, and also other publications have reported effects of polyphenol-containing drinks on plaque formation without referring to its methylxanthine content (Hannig et al. 2009). Whilst there may also be a role for caffeine in combating dental caries (Strålfors 1967), it is likely that methylxanthines and polyphenols may have an effect on dental caries by means of separate mechanisms, suggesting that a combined application may be more beneficial, although more research is necessary to confirm this. The strong inhibition of the metabolic activity of anaerobic bacteria by fluoride in wastewater treatment (Ochoa-Herrera et al. 2009) may well prove to be another decisive factor for the promotion of theobromine- and polyphenol-containing toothpaste in the near future.

2.7 Therapeutic Applications

Theobromine is currently not in use as a medicinal drug. However, *Stedman's Medical Dictionary* (Stedman's Medical Dictionary 1976) describes theobromine as "used as a diuretic, myocardial stimulant, dilator of coronary arteries, and smooth muscle relaxant" and according to Landau (1986), theobromine was used to treat arteriosclerosis and some peripheral vascular diseases, whilst Reynolds (1993) added angina pectoris and hypertension to this list. Rall (1980), however, mentions that it has almost disappeared from the medical scene owing to its low effectiveness in its pharmacological actions compared with caffeine and theophylline, and whilst Tarka (1982) wrote that there was no therapeutic use for theobromine, Simons et al. (1985) mentioned its use in antiasthma medication.

Recent research, however, has identified theobromine as a PARP-1 inhibiting (Geraets et al. 2006), dental enamel strengthening (Sadeghpour 2007) and antitussive (Usmani et al. 2005) agent (see earlier), suggesting there is still a future for theobromine as a medicine, preventative or curative.

3 Caffeine

Unlike theobromine, the effects of caffeine have been extensively investigated. Absorption of caffeine is rapid and complete following oral administration, though in the presence of sugar, absorption is slower but still complete (Yesair et al. 1984), and the maximum blood plasma concentrations (peak plasma time) are reached

within 1 h (James 1991). Indeed, after oral administration of 72 mg caffeine, Mumford et al. (1994) found an onset of subjective effects at 21 min (10–45 min) followed by a caffeine peak plasma time at 30 min after treatment. By means of its adenosine receptor antagonistic properties, caffeine stimulates the CNS and increases blood pressure, respiration, lipolysis, renin and catecholamine release, urine output, and intestinal peristalsis (Landau 1986; James 1991).

Consumption of excessive amounts (more than 1 g/day or more than ten cups of strong coffee per day) can result in tachycardia, dyspepsia (disturbed digestion, decreased appetite, oppressive feeling in the stomach and unpleasant taste), irritability and insomnia, also referred to collectively as "caffeinism" (Landau 1986). Other publications have described symptoms following the intake of high doses of caffeine as "signs and symptoms indistinguishable from those of anxiety neurosis", and nervousness, irritability, tremulousness, occasional muscle twitching, insomnia and sensory disturbances (Tarka 1982) and "a variety of unpleasant subjective states including anxiety, dysphoria and depression" (Mumford and Holtzman 1991).

As a psychostimulant, caffeine increases feelings of energy (more alert, less tired, etc.) and improves other aspects of mood, and enhances psychomotor and cognitive performance when taken in amounts consumed in coffee and tea (Rogers and Dernoncourt 1998; also reviewed in James 1991). Because caffeine reverses overnight caffeine-withdrawal symptoms, which include headache and lethargy (reviewed in Smit and Rogers 2007), it is a powerful ("negative") reinforcer in learned behaviour as indicated, for example, by its ability to increase flavour preference (Rogers et al. 1995; Yeomans et al. 1998). It is this ability which is thought to lie at the heart of the fact that coffee and tea are the world's most popular and widely consumed drinks despite their innate bitterness. Because doses as low as 12.5 mg caffeine have shown behavioural effects (Smit and Rogers 2000), and because such amounts are present in easily consumable portions of chocolate (despite their much higher presence in tea and coffee; see Smit and Rogers 2001), one can only assume that caffeine in chocolate has pharmacological activity, and that caffeine reinforcement could contribute to our liking for chocolate.

4 Biogenic Amines

Cocoa and cocoa products contain biogenic amines (e.g. PEA, tyramine, tryptamine and serotonin) and their precursors (phenylalanine, tyrosine and tryptophan) in fairly high concentrations, which increase during fermentation of the cocoa beans, and decrease during roasting and alkalisation (Ziegleder et al. 1992). In general, these concentrations are irrelevant in healthy people, since biogenic amines are metabolised by the monoamine oxidase (MAO) enzymes in the mucosa of the small intestine, and in the liver and kidneys (Askar and Morad 1980). Because of the endogenous abundance of MAO enzymes, "even the intraduodenal injection of amines in the absence of enzyme inhibition would be unlikely to lead to their absorption and appearance in systemic blood unless the amount was

sufficiently large to swamp the deaminating mechanisms" (Marley and Blackwell 1970). The effects of biogenic amines are therefore only expressed in people with an MAO deficiency, as has been suggested for migraine sufferers (Marley and Blackwell 1970), and in patients receiving medication containing MAO inhibitors (Askar and Morad 1980). These effects, however, can include headaches, increased blood pressure and even a life-threatening "amino shock" (Askar and Morad 1980). Realistically, these adverse effects would presumably lead to the avoidance of chocolate rather than provide an explanation for cravings for chocolate, yet their endogenous biological function may have provided an alleged basis for any wrongfully presumed positive effects. The biogenic amines considered in the following sections have been discussed in the scientific and popular media in this respect.

4.1 Phenylethylamine

2-Phenylethylamine, or ß-phenylethylamine (PEA), is the basic molecule or structure for all compounds that make up the PEA family. This includes the stimulant and hallucinogenic substances amphetamine and mescaline, and the endogenous neurotransmitters dopamine, adrenalin and noradrenalin (Passmore and Robson 1970). Although it has been assumed that chocolate contains large amounts of PEA (e.g. 6 mg/100 g according to the British Food Manufacturing Industries Research Association, cited in Sandler et al. 1974), more recent works suggest much smaller amounts (Koehler and Eitenmiller 1978; Ingles et al. 1985; Hurst and Toomey 1981, with a maximum observed concentration of 0.66 mg/100 g for one particular (milk) chocolate sample – Hurst and Toomey 1981).

Endogenously, PEA occurs in minute quantities (single nanograms per gram of nervous tissue) in the mammalian brain, where it is synthesised by decarboxylation of phenylalanine, almost certainly in dopaminergic neurones. It appears to coexist in the brain with dopamine, and is proposed to be a modulator of catecholamine neurotransmission, though it is rapidly metabolised by MAO type B (Paterson et al. 1990).

Although low levels of endogenous PEA have been linked to depression and high levels have been linked with mania, the evidence for this is mixed and inconclusive (Davis and Boulton 1994). Even so, Liebowitz and Klein (1979) identified an affective disorder involving atypical depression and attention-seeking behaviour ("hysteroid dysphoria") and linked this to an abnormal regulation of PEA. Whilst the authors did not refer to any published evidence, they claimed that "depressed, hysteroid dysphorics often binge on chocolate, which is loaded with phenylethylamine", and that the production of PEA is "stimulated by positive life events". Moreover, PEA has been linked to the euphoric feelings that are part of courtship and sexual activity, mainly on the basis of animal experiments where PEA was injected into the brain (Kohl and Francoeur 1995, after Liebowitz 1983; see also Crenshaw 1996). This, in combination with the notion that PEA is the basic structure of all amphetamines, has led the popular media to link PEA with romance, love and sex, branding PEA a "love drug", making chocolate a "sex substitute".

Obviously, oral consumption and cerebral injection are entirely different modes of administration, and the idea that people eat chocolate to feel "sexier" or more "sensual" because eating chocolate raises endogenous PEA is simply a myth. However, overlooking this distinction may have been used as a convenient tool for the popular media to promote chocolate as a "sex substitute", a message further reinforced when a calculation error resulted in suspiciously high PEA concentrations in chocolate (Hamilton 1992; a value of 660 mg/100 g chocolate miscalculated by a factor of 1,000 from either Table 9 in Hurst and Toomey 1981 or from Table 3 in Hurst et al. 1982, same data). Note that PEA is still freely used to commercially promote the sales of PEA as a nutraceutical, e.g. http://www.americannutrition.com/store/Nootropics.html, accessed 6 August 2009.

On the bais of the evidence available, it is very doubtful that oral intake of PEA causes any beneficial psychopharmacological effects. Indeed, when assessing the effects of a large variety of synthesised amphetamines, administered (usually orally) in various doses, Shulgin and Shulgin (1991) were surprised to find that only PEA did not induce any subjective effects, either orally (200–1,600 mg) or intravenously (25–50 mg). Clearly, PEA needs side groups to function as an active amphetamine, and these findings further substantiate the "PEA myth" of chocolate.

4.2 Tyramine

Tyramine is present in a variety of foods, but its levels in chocolate are relatively low and are akin to those of PEA (Koehler and Eitenmiller 1978; Ingles et al. 1985; Hurst and Toomey 1981). Like PEA, tyramine has also been implicated in migraine attacks, and in the "cheese reaction" (*tyros* is Greek for "cheese"; Passmore and Robson 1970): prescribed in the late 1950s and the 1960s for depression and hypertension, MOA inhibitors made patients sensitive to the toxic effects of tyramine, found in some cheeses in relatively high amounts – up to 62.5 mg/100 g was measured by Ingles et al. (1985) and ten Brink et al. (1990). Symptoms of the "cheese reaction" included hypertensive crisis and severe headache, sometimes even leading to intracranial bleeding or cardiac failure (Joosten 1988). However, there appears to be no published evidence suggesting any beneficial effects of tyramine on mood and behaviour.

4.3 Serotonin and Tryptophan

As a neurotransmitter in the CNS and the peripheral nervous system, serotonin (5-hydroxytryptophan) plays an important role in the regulation of mood and behaviour (Young 1993). Although it has been identified in a range of foods, bananas, pineapples and chocolate contain somewhat higher than average concentrations (2.5, 4.2 and 2.7 mg/100 g – averages calculated from Smith 1981; Marley

and Blackwell 1970; Hurst and Toomey 1981), although the highest concentrations of serotonin have been found in walnuts (55 mg/100g; Smith 1981). Note that as for all biogenic amines, also serotonin is metabolised rapidly after oral intake, and consumption of foods containing serotonin will not directly affect brain levels of serotonin. This fits with the observation that cravings for walnuts are not common, certainly when compared with the prevalence of cravings for chocolate.

As a *precursor* of serotonin, the amino acid tryptophan is *not* prone to deamination. However, large pharmacological doses of tryptophan (much larger than our normal dietary intake of 1–1.5 g/day) can be an effective antidepressant (Young et al. 1986), which is consistent with the idea that a deficit in serotonergic activity is important in the vulnerability to depression (Maes and Meltzer 1995). Likewise, tryptophan has shown improvements in depressive symptoms in seasonal affective disorder (McGrath et al. 1990) and premenstrual syndrome (Steinberg et al. 1986), and people prone to depression show deteriorated mood following the administration of tryptophan-depleted mixtures of amino acids (Young et al. 1986; Benkelfat et al. 1994). Although these studies suggest a clear role for the serotonergic system in the cause of depression, altered brain levels of tryptophan and therefore serotonin are not expected to occur when tryptophan is consumed through the regular diet owing to competition for uptake into the brain with other large neutral amino acids (Young 1993; Rogers 1995). It is therefore extremely unlikely that any mood changes that may arise from the consumption of chocolate are caused by its tryptophan content.

5 Anandamide

Anandamide (arachidonylethanolamide), an endogenous ligand for the cannabinoid receptor that binds competitively to brain cannabinoid receptors, has been identified in minute concentrations (0.05 μg/g) in chocolate, where this compound is contained in the cocoa solids, as its presence was not confirmed in white chocolate (di Tomaso et al. 1996). Unsubstantiated, the authors suggest that anandamides present in food might "heighten sensitivity and produce euphoria" and in doing so, intensify the orosensory effects of chocolate. However, the bioavailability of anandamide is no more than 5% (Di Marzo et al. 1998). Note also that Δ^9-tetrahydrocannabinol, the main psychoactive compound in cannabis, showed a noticeable "high" in human volunteers at doses as low as 18.77 μg/kg body weight (equivalent to 1.3 mg for a 70-kg person) (Perez-Reyes et al. 1973). It then follows that, even if one were to make the generous assumption that anandamide is as bioavailable, stable and potent (magnitude of drug effect) as Δ^9-tetrahydrocannabinol, a blood plasma concentration of 18.77 μg/kg body weight can only be achieved by consuming 25 kg chocolate in a single sitting – a most uncomfortable, if not impossible task with potentially lethal consequences. This therefore also contradicts the suggestion of di Tomaso et al. that their findings "point to an unexpected link between non-drug craving and the endogenous cannabinoid system". The fact that a cannabis user tried to convince the court of having consumed "a massive

amount of chocolate" in defence against the accusation of using and supplying cannabis (this involved a positive routine urine test; Tytgat et al. 2000) only confirms how the discovery of di Tomaso et al. resulted in yet another myth about our liking and cravings for chocolate.

6 Salsolinol and Tetrahydro-ß-carbolines

Salsolinol (SAL) and tetrahydro-ß-carbolines (THBCs) are neuroactive alkaloids generated endogenously following the consumption of alcohol through a reaction between the primary alcohol metabolite acetaldehyde and dopamine to create SAL,[1] or between acetaldehyde and indoleamines (e.g. serotonin, tryptamine, tryptophan) to create THBCs (Quertemont et al. 2005). Both SAL (Haber et al. 2002) and THBCs (Myers 1989) have been implied as an important factor in alcoholism, and investigated as such (Quertemont et al. 2005).

Additionally, SAL and THBCs have been identified in chocolate: SAL has been found in milk and dark chocolate and cocoa at 5, 20 and 25 mg/kg respectively; Melzig et al. 2000), whilst THBCs were identified in comparable though slightly lower amounts (1.4, 5.5 and 3.3 mg/kg respectively; Herraiz et al. 1993). In part driven by their implication in alcoholism, SAL (Melzig et al. 2000) and THBCs (Herraiz 2000) have independently been named as potentially involved in cravings for chocolate.

Again, also here, a role for SAL and THBCs in the cause of chocolate cravings would require that their consumption results in raised blood plasma levels of these compounds. Unfortunately, the literature is not clear whether this occurs or not. Even if they could be freely absorbed, THBCs are also mild MAO inhibitors (see Sect. 4 for MAO inhibition), potentially amplifying the effects of biogenic amines in chocolate and thereby contributing to migraines following the consumption of THBCs (Baker et al. 1987 in Herraiz 2000; Herraiz and Chaparro 2006) or SAL (Heikkilla et al. 1971 in Melzig et al. 2000) in chocolate. Furthermore, although SAL reportedly shows positive effects on heart rate and muscle contractions (Sokolova et al. 1990 and Chavez-Lara et al. 1989, respectively, in Melzig et al. 2000), again the route of administration is not clear. Finally, a particular THBC has been found in the tubers of the South American maca plant (*Lepidium meyenii* Walp.). Whilst this plant has been ascribed various therapeutic benefits, and is used by athletes as an alternative to anabolic steroids (Brack Egg 1999 in Piacente et al. 2002), it is perfectly possible that these effects are related to one or more of the other maca constituents, especially glucosinolates.

On the basis of the evidence available, it is unlikely that chocolate cravings can be induced by SAL or THBCs. Like PEA, tyramine and anandamide, SAL and

[1]SAL is the most widely researched example of a tetrahydroisoquinoline; tetrahydroisoquinolines are formed from acetaldehyde and catecholamines (Quertemont et al. 2005)

THBCs may have to be added to the ever-growing list of myths surrounding this topic as there is no direct evidence indicating biological activity through oral intake of either substance.

7 Magnesium

Chocolate has been mentioned as a relevant source of dietary magnesium (Gibson 1990). Indeed, according to some publications, it has one of the highest magnesium levels of all foods listed (Seelig 1989; Rozin et al. 1991). Moreover, magnesium therapy has been claimed to reduce premenstrual tension (Abraham 1980) and to reduce chocolate cravings in women on hormone replacements (Roach 1989; A. Weil, personal communication). Although this appears to indicate an explanation for "why women crave for chocolate at that particular time of the month", the following findings need to be taken into consideration before any such claims can be made.

First of all, dark chocolate contains 90–100 mg magnesium/100 g, whilst milk chocolate magnesium levels are slightly lower, at 43–50 mg magnesium/100 g (FSA 2002; Souci et al. 1986). Although chocolate may have the potential to contribute to the dietary intake of magnesium, and to even counteract magnesium deficiency, other foods contain similar or even much larger amounts of magnesium, for example Brazil nuts (410 mg/100 g), roasted and salted cashew nuts (250 mg/100 g), peanuts (210 mg/kg) and All-Bran cereal (240 mg/100 g) (FSA 2002). Interestingly, cravings for these foods do not appear to be very common in sufferers from premenstrual tension, as is confirmed by the observation that chocolate is the main target in female food cravings (Hill and Heaton-Brown 1994; Rodin et al. 1991), followed by ice-cream (Rodin et al. 1991). Indeed, "the food cravings reported... were hunger-reducing, mood-improving experiences, directed at wanting to consume highly pleasant tasting food" (Hill and Heaton-Brown 1994).

Therefore, despite speculated associations between changes in mood, food preferences and the menstrual cycle (Bancroft et al. 1988; Wurtman and Wurtman 1989), there is no reliable evidence to suggest that magnesium-deficient people show an increased craving or liking for chocolate.

8 Conclusions and Considerations

Most of the pharmacologically active substances present in chocolate that have been highlighted by both scientists and the popular media do not exude an effect in man owing to extremely low concentrations, the inability to cross or even reach the blood–brain barrier, or other inabilities that may at times have been conveniently ignored in order to justify a message that appeals to the general public. Of all constituents proposed to play a role in why we like chocolate over and above its

innate appeal as a sweet and creamy tasting food (Drewnowski and Greenwood 1983), caffeine appears to provide the clearest evidence, based on effects found with ecologically relevant doses (Smit and Rogers 2001). Although theobromine is a promising "candidate", synergistic or detrimental interactions as found in animal research (Heim et al. 1971; He et al. 2009) cannot be ruled out and need to be investigated with doses relevant to generally consumed amounts, as well as the possibility that some people are much more sensitive to the effects of theobromine than others (Mumford et al. 1994; Ott 1985). Research ought to include caffeine as a comparative agent, and underlying mechanisms need to be further explored, especially in the case of theobromine as an antitussive (Usmani et al. 2005) or as a dental enamel protective (Sadeghpour 2007) agent. In animals, theobromine is much better researched, despite this being fairly limited to toxicology studies. Additionally, a range of case studies of animal poisoning point out the dangers of chocolate and other cocoa-containing products to a wide range of animal species. Finally, doping control and therefore potential disqualifications are an issue in animal racing sports. However, the effects found in animals, whether they be of a toxicological, behavioural or other nature, cannot necessarily be translated to the human system (Tarka et al. 1979; Miller et al. 1984).

Clearly, the focus of interest regarding the effects of theobromine has charged over time, and new developments are promising an interesting future for a much underresearched substance. Hence, theobromine is in need of further investigation, and the following points need to be addressed:

1. A theobromine-to-caffeine affinity ratio for adenosine receptors of possibly 1:10 is not compensated for the estimated 10:1 theobromine-to-caffeine prevalence ratio in chocolate in terms of its mood, mental performance, or subjective effects.
2. It appears some individuals may be sensitive to the subjective effects of theobromine, though this needs validating, and a theoretical basis for this needs to be established.
3. Although some interactive effects of caffeine and theobromine have been observed, and although the effects of low doses of caffeine and of relevant caffeine–theobromine combinations on mood and performance have been found, effects of the individual components in relation to their combination have not yet been reported.
4. Although antitussive and enamel-strengthening effects of theobromine may have been found, similar effects of cocoa or chocolate ingestion, or comparative effects with caffeine and maybe other methylxanthines need to be investigated, in part to gain better insight into the possible mechanisms involved.

Note that although the methylxanthines in chocolate appear to represent its pharmacological activity (Smit et al. 2004), the list of minor chocolate constituents presented here is not exhaustive, nor does it address potential interactive effects between compounds that are not explained by their individual effects (Perez-Reyes et al. 1973). Moreover, although pharmacological activity *can* play a role in the liking for a food (see Smit and Blackburn 2005 for the combination of caffeine and theobromine as an example of their role in the liking for chocolate), this does not

translate into *cravings* for such a food. Indeed, Michener and Rozin (1994) showed that only the sensory experience of a food, and not the pharmacologically active constituents, could fulfil such cravings. Note also that cravings for chocolate are usually directed at milk chocolate, containing lower quantities of the active constituents, and that people rarely describe strong urges for the consumption of coffee and tea, even when caffeine intake is reduced because of changes in daily routine (Rogers and Dernoncourt 1998). Finally, unlike chocolate, caffeine intake is rarely resisted ("dietary restraint"). Taking these general observations and experimental findings into account, we must seek the most plausible explanation for the existence of cravings for chocolate and even chocolate "addiction" in a culturally determined ambivalence towards chocolate (Cartwright and Stritzke 2008; Smit and Rogers 2001; Rogers and Smit 2000), and not in a role for any chocolate constituents.

Summarising, despite the assumption of being a behaviourally inert substance, theobromine has shown a range of interesting effects, both in man and in other animal species. Novel findings may have caused a renewed interest in this caffeine-related compound, and much is yet to be clarified. Also, with regard to my personal interest in the psychopharmacological effects of chocolate, I can only conclude that the last word on theobromine has not yet been heard.

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