The vascular effects of trace amines and amphetamines

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Abstract

Trace amines, including tyramine, β-phenylethylamine (β-PEA), tryptamine and octopamine, are biologically active amines mostly based on phenylethylamine, occurring in the body in trace amounts. They are a diverse group of naturally occurring and synthetic amines, which are also found in the diet and in herbal plants, such as ephedrine and cathinone. They include amphetamine and its analogues, such as MDMA (‘ecstasy’), and synthetic proprietary sympathomimetic agents such as phenylpropanolamine and pseudoephedrine. On the vascular system they cause vasoconstriction and a rise in blood pressure. This effect is the basis of their use as nasal decongestants. For over 50 years, they have been assumed to be indirectly acting sympathomimetic amines, their responses being due to the release of noradrenaline from sympathetic neurones. There are, however, results that suggest that this is not their only mechanism of action and that they may also exert direct vascular effects independent of a noradrenergic mechanism. Recently, a group of novel trace amine-associated receptors (TAARs) have been cloned and identified in the brain and peripheral tissues including blood vessels. Trace amines bind to these cloned receptors and it is suggested that their vasoconstrictor effects can in part be attributed to this mechanism. This review describes the cardiovascular pharmacology of this diverse group of amines, their structures and uses and their endogenous synthesis and metabolism. The review also considers their clinical relevance as constituents of the diet, as therapeutic agents (ritodrine, phenylpropanolamine, and pseudoephedrine) and as drugs of abuse (amphetamine, ‘ecstasy’) and their mechanisms of action.

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1. Introduction

Trace amines are defined as biologically active amines occurring in the body in trace amounts. They include tyramine, β-phenylethylamine (β-PEA), tryptamine and octopamine (Fig. 1). They are also found in the diet, derived from plants, bacteria and fungi (Burchett & Hicks, 2006). Foods rich in tyramine and β-PEA include cheeses, red wine, fermented foods such as sausages, and cocoa-containing foods such as chocolate (Branchek & Blackburn, 2003). Trace amines are present in very small nanomolar concentrations in the mammalian brain (Borowsky et al., 2001; Zucchi et al., 2006; Burchett & Hicks, 2006). They are structurally and functionally related to the catecholamines and there are a large number of synthetic analogues, such as the amphetamines (Bunzow...
et al., 2001; Branchek & Blackburn, 2003; Lindemann & Hoener, 2005). Trace amines are primary amines generated directly by enzymatic decarboxylation of their respective precursor amino acid (Boulton & Dyck, 1974; Tallman et al., 1976; Borowsky et al., 2001; Bunzow et al., 2001; Lindemann & Hoener, 2005).

2. Dietary sources of trace amines

Tyramine occurs in many fermented foods, reaching substantial concentrations in products such as sausages (~200 mg/kg) (Suzzi & Gardini, 2003) and goat’s (2000 mg/kg) (Bonetta et al., 2008) and Dutch cheeses (300 mg/kg) (Komprda et al., 2008). Probiotic foods are also a source of tyramine and β-PEA, generated by the high levels of lactic acid-producing bacteria such as Lactobacillus, Lactococcus and Enterococcus species (Marcobal et al., 2006). These are said to be ‘friendly’ bacteria, displacing harmful bacteria of the gut flora (Taylor & Mahenthralingham, 2006) and combating infectious diarrhoea (Fedorak & Madsen, 2004). The commercial promotion of probiotic drinks and yoghurts is also based on a perceived ability to alleviate bloating and abdominal distension (Houghton & Whorwell, 2005). This contention is supported by clinical evidence that bloating (Kim et al., 2003) and flatulence (Nobaek et al., 2000; Kim et al., 2005) associated with irritable bowel syndrome are reduced by probiotic bacterial supplements. Cocoa-based foods, such as chocolate, also contain tyramine and β-PEA. The levels of β-PEA in cocoa have been reported to range from 1.8 mg/kg (Ziegleder et al., 1992) to 22 mg/kg (Baker et al., 1987). This wide range has been attributed to the difficulties in extraction of the amines from the complex food matrix.

Fig. 1. Chemical structures of trace amines, amphetamines and sympathomimetic amines.
and to the fact that the amines are generated by decarboxylation of the parent amino acid by different levels of roasting of the fermented cocoa beans (Granvogt et al., 2006).

Tyratmine is also found in tobacco (Slocum et al., 1989) and in the cocoa that is added to cigarette tobacco to enhance the flavor. Tyratmine and β-PEA in cigarettes form reaction products during combustion which have monoamine oxidase (MAO) inhibitory properties. These MAO inhibitors may contribute to the addiction associated with cigarette smoking (Rambali et al., 2002). In smokers, brain (Fowler et al., 2003), lung and heart (Logan & Fowler, 2005; Fowler et al., 2005) levels of MAO A and B are reduced by up to 40%. Additionally, several other compounds found in cigarette smoke have been shown to inhibit MAO enzymes (Fowler et al., 2003). Since MAO metabolizes these trace amines (Section 4), inhibition of MAO by cigarette smoking has the potential to increase endogenous levels of trace amines. A daily consumption of 25 cigarettes can result in an intake of approximately 12.5 mg and 10.4 mg, respectively of β-PEA and tyratmine, assuming 100% of the amine is transferred to the smoke. These levels are relatively high compared with daily dietary intake and it must also be remembered that the inhalation route avoids first-pass metabolism in the liver, unlike consumption in foods (Rambali et al., 2002).

Tryptamine may also be described as a trace amine and is also found in the diet in vegetables such as tomatoes with levels of approximately 222 µg/g of dry weight (Ly et al., 2008). Octopamine is the main neurotransmitter in invertebrates and replaces noradrenaline as the neurotransmitter in the sympathetic nervous system (Roeder, 1999). Octopamine also occurs in some foods and food supplements, such as Bitter Orange (Citrus aurantium), which has been marketed as a dietary supplement for appetite suppression. The major alkaloid found in C. aurantium is synephrine with smaller amounts of octopamine and tyratmine (Putzbach et al., 2007). These products were introduced for weight loss when ephedra-containing (Ephedra sinica) dietary supplements were banned by the Food and Drug Administration after they were associated with strokes and heart attacks (Haller & Benowitz, 2000). Synephrine and octopamine appear to promote weight loss by selectively activating β3-adrenoceptors on lipocytes to stimulate lipolysis and promote thermogenesis (Carpene et al., 1999).

3. Amphetamines and other synthetic sympathomimetic amines

The forerunner of all synthetic amphetamines is ephedrine, the active constituent of Ephedra, which in traditional Chinese medicine is known as Ma huang. Ma huang has been used as an herbal remedy for asthma, hay fever and the common cold for 5000 years (Abourashed et al., 2003). The primary species of the Ephedra family used as the source of ephedrine is Ephedra sinica (Gurlley et al., 1998), which has a total alkaloid content of 1–3% by dry weight and ephedrine constitutes 40–90% of this alkaloid content. Ephedrine is a stimulant and has been widely used for weight loss and in sports to enhance performance. The potential for serious adverse effects from ephedrine use and the high profile deaths of several prominent sportsmen, have led to it being classed as a banned substance by the International Olympic Committee (Shekelle et al., 2003). Accumulating evidence of adverse effects and deaths related to ephedra, resulted in the FDA banning the sale of ephedra-containing supplements on April 12th 2004. Ephedrine remains available in over-the-counter cough and cold remedies. An emerging problem with ephedrine is that it is a chemical source for the manufacture of more active stimulant sympathomimetics such as methamphetamine (Barker & Antia, 2007).

Another sympathomimetic amine that was first synthesised in 1912 (Calliess, 1912) and has been widely used as a proprietary medicine for weight loss (Weintraub, 1985) is phenylpropanolamine. It has pressor actions and one of its earliest clinical uses was as a nasal decongestant (Forshay et al., 1983), due mainly to vasoconstriction in the nasal mucosa. Phenylpropanolamine has also been used for weight loss (Lasagna, 1988), an effect attributed to both appetite suppression in common with amphetamine and to increased thermogenic lipolytic activity of brown adipocytes (Wellman & Sellers, 1986), presumably through β3-adrenergic receptor stimulation (Collins & Survit, 2001).

Amphetamine is a sympathomimetic amine with important anorectic and central stimulant properties. Nowadays, it is mainly used in the treatment of narcolepsy and attention deficit hyperactivity disorders (ADHD) (Seiden et al., 1993; Seeman & Madras, 1998). Amphetamine and the synthetic derivative, methamphetamine, are potent psychostimulants causing wakefulness and euphoria that can lead to abuse and addiction (Anglin et al., 2000). Methamphetamine is the most widely used illegal drug in the world according to the United Nations. In North America, South East Asia, Australia and Japan, methamphetamine abuse causes major public health problems (Iversen, 2006). 3-4-methylenedioxy-methamphetamine (MDMA, ‘ecstasy’) is an amphetamine-like drug of abuse, which is the second most commonly taken drug in the U.K., after cannabis (Condon & Smith, 2003). There is a marked worldwide increase in popularity of ecstasy use (Landry, 2002), which is attributed to its positive effects on mood and well being and perceived safety (Peroutka et al., 1988). In recent randomised, blinded trials, ecstasy was reported to induce marked euphoria, friendliness, closeness and empathy in human volunteers given single doses in a controlled laboratory setting (Camí et al., 2000; Harris et al., 2002). MDMA became widely available in the USA when MDA (3-4-methylenedioxyamphetamine) was declared an illegal drug. MDA is a metabolite of MDMA, about 7% appearing in the urine. DOM (2,5-dimethoxy-4-methyl-amphetamine) is another related drug of abuse with marked hallucinogenic properties (Wills, 2002) (Fig. 1).

The chewing of khat (or qat) leaves (Catha edulis Forsk.) in East Africa and The Yemen forms a deep-rooted social and cultural function (Al-Motarreb et al., 2002a). This habit has spread to ethnic communities in Britain including Somali Communities in South Wales and London (Griffiths, 1998). The pleasure derived from khat chewing is attributed to the euphoric actions of its content of S-β-cathinone, a sympathomimetic amine with properties described as amphetamine-like (Kalix & Braendén, 1985). Like MDMA and amphetamine, cathinone exerts pronounced behavioural effects of euphoria, excitability, hyperactivity and restlessness with hyperthermia (Kalix, 1984; Kalix & Braendén, 1985).

Methylenidate (MPH, Ritalin®) is an amphetamine-related agent that is the most common drug used for the treatment of attention deficit hyperactivity disorders, which is the most common behavioural disorder of childhood (Wilens et al., 2002; Iversen, 2006). Ritalin® is a psychomotor stimulant with sympathomimetic actions which enhances the release of noradrenaline and blocks the re-uptake of noradrenaline and dopamine (Hendley et al., 1972). It is also effective for the treatment of narcolepsy (Littner et al., 2001) and as an antidepressant in elderly cancer patients (Rozans et al., 2002). Unfortunately, Ritalin also has reinforcing effects particularly when taken intravenously or when snorted, which can result in addiction and abuse (Parran & Jasinski, 1991).

4. Synthesis and metabolism

Endogenous trace amines are synthesised in the body by the decarboxylation of their respective precursor amino acids using aromatic ω-amino acid decarboxylase (AADC) (EC 4.1.1.28) (Berry, 2004). β-Phenylethylamine is synthesised from ω-phenylalanine, while p-tyramine is synthesised from ω-tyrosine. p-Octopamine is derived from p-tyramine via dopamine-β-hydroxylase (DBH) (EC 1.14.17.1.) (Fig. 2). m-Tyramine and m-octopamine are also synthesised and found in the mammalian brain (Berry, 2004). Tryptamine is synthesised from α-tryptophan by the action of AADC and levels equivalent to tyramine and β-PEA are found in the brain (Ruddick
Trace amines are metabolized in the mammalian body via monoamine oxidase (MAO; EC 1.4.3.4) (Berry, 2004) (Fig. 2). MAO is an intracellular enzyme found tightly bound to the outer membrane of mitochondria. It deaminates primary and secondary amines that are free in the neuronal cytoplasm but not those bound in storage vesicles of the sympathetic neurone. The reaction of MAO with its substrate requires molecular oxygen and water and results in the generation of the aldehyde, ammonia and hydrogen peroxide:

\[
\text{R}/\text{C}_0\text{CH}_2\text{NH}_2 + \text{O}_2 + \text{H}_2\text{O} \rightarrow \text{R}/\text{C}_0\text{CHO} + \text{NH}_3 + \text{H}_2\text{O}_2
\]

Two isozymes of MAO having different substrate specificities and locations have been identified. MAO-A predominates in the stomach, intestine and placenta and is found in sympathetic neurones as well as extraneuronally. The preferred substrates for MAO-A are polar aromatic amines such as noradrenaline, adrenaline, serotonin (5-hydroxytryptamine, 5-HT) and octopamine. MAO-B is found in platelets and serotonergic neurones and predominates in the brain. MAO-B selectively deaminates non-polar aromatic amines such as \(\beta\)-phenylethylamine, and dopamine is a relatively selective substrate (Broadley, 1996).

The aldehyde derivatives of MAO metabolism are rapidly converted to \(p\)-hydroxyphenylacetic acid by the action of aldehyde dehydrogenase (ADH) (EC 1.2.1.3.). A further route of metabolism in vivo is via N-methylation by non-specific N-methyl transferase (NMT) (EC 2.1.1.49) or by phenylethanolamine N-methyltransferase (PNMT) (EC 2.1.1.28), found in the adrenal medulla, to form the corresponding secondary product.

Fig. 2. Synthetic and metabolic pathways for endogenous and exogenously administered trace amines and sympathomimetic amines. AADC, \(\alpha\)-aromatic amino acid decarboxylase; ADH, aldehyde dehydrogenase; DBH, dopamine-\(\beta\)-hydroxylase; L-DOPA, \(L\)-dihydroxyphenylalanine; MAO, monoamine oxidase; NMT, N-methyl transferase; PNMT, phenylethanolamine N-methyltransferase. Also shown for comparison are the synthetic and metabolic pathways for the neurotransmitter, noradrenaline. Although not shown, noradrenaline and the other trace amines are also subjected to N-methylation by PNMT and NMT.
amines (Saavedra et al., 1973, 1974). These synthetic pathways also
generate the sympathetic neurotransmitter, noradrenaline, which also
undergoes metabolism via MAO, ADH and PNMT (Fig. 2).

A further group of copper-containing enzymes that may metab-
olize trace and dietary amines are the semi-carbazide-sensitive amine
oxidases (SSAO) (EC 1.4.3.6). SSAO deaminates aromatic and aliphatic
amines in an identical manner to MAO, also deaminates histamine, is
not inhibited by MAO inhibitors such as clorgyline, but is inhibited by
semi-carbazide. It has been found in large amounts in vascular tissue,
such as the rat aorta (Lyles, 1994; Magyar et al., 2001).

MAO present in the intestine and the liver therefore controls the
circulating levels of dietary amines, such as tyramine and β-
phenylethylamine. In individuals taking MAO inhibitors, the ingestion
of tyramine-containing foods results in large amounts of tyramine
reaching the peripheral tissues. Their established mechanism of action
(Section 6) is that they are indirectly acting sympathomimetic amines,
whereby they enter sympathetic neurones and release noradrenaline,
which causes α-adrenoceptor-mediated vasoconstriction and a
transient hypertension. Individuals on MAO inhibitor treatments
have been known to experience a life-threatening hypertensive crisis
which in certain individuals has been fatal due to an intracranial
haemorrhage. This is known as the “cheese effect”. It is also relevant to
other sympathomimetic amines contained in over-the-counter cough
and cold remedies as decongestants, such as phenylpropanolamine
and pseudoephedrine. Thus, MAO inhibitors potentiate the peripheral
effects of indirectly acting sympathomimetic amines. It is not often
realized, however, that this potentiation occurs irrespective of
whether the amine is a substrate for MAO. An α-methyl group on
the side chain, as in amphetamine and ephedrine, renders the amine
immune to deamination so that they are not metabolized in the gut.
Similarly, β-PEA would not be deaminated in the gut as it is a selective
substrate for MAO-B which is not found in the gut. However, MAO
inhibition in sympathetic neurones allows the cytoplasmic pool of
noradrenaline to increase. It is this pool that is released by indirectly
acting sympathomimetic amines and their responses are therefore
potentiated irrespective of whether they are deaminated by MAO

Brain levels of endogenous trace amines are several hundred-fold
below those for the classical neurotransmitters noradrenaline,
dopamine and serotonin but their rates of synthesis are equivalent
to those of noradrenaline and dopamine and they have a very rapid
turnover rate (Berry, 2004). Endogenous extracellular levels of
trace amines measured in the brain are in the low nanomolar range.
These low concentrations arise because of their very short half-life,
lack of storage and the fact that they originate by diffusion
mechanisms following their intracellular synthesis (Berry, 2004).
Peripheral levels will be considered in more detail in Section 8.

5. Vascular responses to trace amines

5.1. Blood pressure

Oral administration to humans of tyramine (Peatfield et al., 1983),
ephedrine (Berlin et al., 2001), cathinone (Brenneisen et al., 1990)
and the synthetic amines, phenylpropanolamine (Salerno et al.,
2005), amphetamine (Iversen, 2006), methylphenidate (Godfrey,
2009) and MDMA (“ecstasy”) (Mas et al., 1999) cause increases in
blood pressure. Khat chewing by human volunteers increases systolic
blood pressure by 20 mm Hg (Ghose, 1984). Intravenous tyramine increases
blood pressure but this effect has been associated with an increase in
cardiac output rather than vasoconstriction. A decrease in total
peripheral resistance has been observed suggesting that there is a
baroreceptor-mediated reflex vasodilatation induced by the initial rise
in blood pressure (Meck et al., 2003). Tyramine infusion to healthy
volunteers caused an increase in systolic but not diastolic blood
pressure, but there was an increase in forearm blood flow (Jacob et al.,
2003). These changes were accompanied by both systemic and forearm
spillover of noradrenaline, indicating that there had been local release of
noradrenaline by the tyramine. Since the increase in blood pressure was
inhibited by the selective α1-adrenoceptor antagonist, doxazosin, it is assumed
that the pressor effect is of cardiac origin rather than through
vasoconstriction (Schafer et al., 1997). The heart rate does not increase
dramatically, suggesting that the cardiac effect is due to positive
inotropy. It has been suggested that any direct increase in heart rate by
tyramine is offset by a compensatory reflex bradycardia via the vagal
nerves. This is supported by the observation that the muscarinic
antagonist, atropine, potentiated the increase in heart rate and blood
pressure induced by tyramine (Schafer et al., 1997). The paradoxical
vasodilation by intravenous tyramine administration and local release
of vasoconstrictor noradrenaline has not been satisfactorily explained.
The local effect of tyramine applied directly via the brachial artery to the
forearm is, as expected, vasoconstriction (Tschanovsky et al., 2002). A possible
explanation for the paradox is that tyramine also releases other
vasodilator autacoids, such as dopamine, levels of which are raised after
tyramine infusion (Jacob et al., 2003). ATP may also be released by
tyramine as it is released endogenously to cause vasodilatation and
regulation of local blood flow by blunting sympathetic vasoconstrictor
activity (Rosenmeier et al., 2004). Tyramine co-releases ATP and noradrenaline
from guinea-pig vas deferens, but this was not attributed to
neuronal release but from a non-neuronal source secondary to
activation of postjunctional α-adrenoceptors by the noradrenaline
(Driessen et al., 1996). A further likely vasodilator autacoid released by
tyramine is nitric oxide. Intravenous infusion of tyramine in conscious
rabbits raised blood pressure and increased hindlimb vascular resis-
tance. These responses were enhanced by the nitric oxide synthase
inhibitor, N-nitro-L-arginine, indicating that there was an opposing
vasodilator effect of tyramine mediated via the release of nitric oxide
(Du et al., 1992).

In animals, intravenously administered tyramine is well known to
cause increases in blood pressure in rats (Day, 1967; Liles et al., 2006;
Khwanuch et al., 2008) (Fig. 3), cats (Burn & Rand, 1958; Day, 1967),
dogs (Woodman & Pannangpetch, 1994) and rabbits (Du et al., 1992). In
the rat, the increase in blood pressure was antagonized by the α-
adrenoceptor antagonist, phentolamine, but not when combined with
propranolol or propranolol and atropine (Khwanuch et al., 2008). The
authors explained this observation by stating that tyramine may have
direct actions which are only revealed when sympathetic reflexes are
inhibited. Other amines, such as phenylpropanolamine (Moya-Huff et al.,
1987), MDMA (“ecstasy”) (O’Cain et al., 2000; McDaid & Docherty,
2001), ephedrine (Liles et al., 2006), methamphetamine (Varner et al.,
2002), amphetamine and β-PEA (Day, 1967) all cause pressor effects in
conscious, anaesthetized or pithed rats. Tyramine, amphetamine and
cathinone cause pressor responses in dogs (Kohli & Goldberg, 1982).

The question is whether these effects are due to vasoconstriction
or as suggested above for humans, they can be attributed wholly or in
part to an increase in cardiac output via a β-adrenoceptor-mediated
positive inotropic effect (Schafer et al., 1997). The pressor response to
ephedrine was partially antagonized by the α-adrenoceptor
and found that resting perfusion pressure was little affected by tyramine (Fig. 5). In rat isolated perfused mesenteric artery bed, we have (Al-Motarreb & Broadley, 2003) and pig coronary arteries (Baker et al., 2007). Tyramine produced equivalent vasoconstrictor responses to amphetamine in rat aorta using ring preparations (Fehler et al., 2008a). Tyramine produced equivalent vasconstriction in aortic preparations from rats, guinea-pigs and rabbits (Maling et al., 1971). We have recently shown that β-PEA and tyramine cause vasconstriction of porcine isolated coronary artery rings (Herbert et al., 2009) (Fig. 4) and rat aortic rings (Fehler et al., 2008a). The contractions of fetal lamb isolated umbilical arteries to 2, 5-dimethoxy-4-methyl-amphetamine (DOM) were antagonized by the 5-HT2 receptor antagonist, ketanserin, suggesting that this amphetamine derivative causes vasconstriction via 5-HT2 receptors (Zhang & Dyer, 1990).

In isolated blood vessels there is no doubt that tyramine and other amines cause vasconstriction (Fig. 4). Tyramine, but not amphetamine, methamphetamine and hydroxyamphetamine, caused vasconstriction in spiral preparations of rat aorta (Krishnamurty & Grollman, 1972). However, more recently we have demonstrated vasconstrictor responses to amphetamine in rat aorta using ring preparations (Fehler et al., 2008a). Tyramine produced equivalent vasconstriction in aortic preparations from rats, guinea-pigs and rabbits (Maling et al., 1971). We have recently shown that β-PEA and tyramine cause vasconstriction of porcine isolated coronary artery rings (Herbert et al., 2009) (Fig. 4) and rat aortic rings (Fehler et al., 2008a). The contractions of fetal lamb isolated umbilical arteries to 2, 5-dimethoxy-4-methyl-amphetamine (DOM) were antagonized by the 5-HT2 receptor antagonist, ketanserin, suggesting that this amphetamine derivative causes vasconstriction via 5-HT2 receptors (Zhang & Dyer, 1990).

In rat mesenteric arteries (Al-Sahli et al., 2001) and rabbit isolated ear arteries (Fitzgerald & Reid, 1994), MDMA potentiated the vasconstrictor responses to noradrenaline, which was presumably due to inhibition of neuronal uptake of noradrenaline by MDMA. Our recent studies showed for the first time that MDMA exerted a direct vasconstrictor action in isolated coronary arteries (Baker et al., 2007) (Fig. 5). Like MDMA and amphetamine, cathinone potentiated the vasconstrictor response to noradrenaline, which was presumably due to inhibition of neuronal uptake of noradrenaline by MDMA. Our recent studies showed that cathinone causes a direct vasconstrictor action in isolated coronary arteries (Baker et al., 2007) (Fig. 5). In rat isolated perfused mesenteric artery bed, we have found that resting perfusion pressure was little affected by tyramine and β-PEA. However, in contrast to other vascular tissues, when perfusion pressure was raised by infusion of the α-adrenoceptor agonist phenylephrine, tyramine and β-PEA caused dose-related falls in perfusion pressure indicative of vasodilatation, rather than vasconstriction (Broadley et al., 2009). Tryptamine, however, caused a direct vasconstrictor response (Anwar et al., 2006, 2008). It is our hypothesis that dietary trace amines cause vasodilatation in the mesenteric vascular bed to increase blood flow to the gastrointestinal tract, thereby aiding digestion and absorption. To provide this increased blood flow, vasconstriction elsewhere diverts blood from other organs to the gut (Broadley et al., 2009).

6. Established mechanism for the vascular effects of trace amines

The accepted mechanism for the responses to trace amines, dietary amines and amphetamines is that they behave as indirectly acting sympathomimetic amines through the release of noradrenaline from sympathetic neurones (Broadley, 1996). There is abundant evidence for the release of noradrenaline by a range of amines. For example, prelabelled [3H]-noradrenaline is released by cathinone from rabbit atria (Kalix, 1983), by tyramine from anococcygeus muscle (McGrath & Olverman, 1978), by MDMA from rabbit isolated perfused and superfused ear arteries (Fitzgerald & Reid, 1994), by dexamphetamine from rat vasa deferentia (Langloh & Trendelenburg, 1987) and by β-phenylethylamine from rabbit atria (Paton, 1975). Indirect sympathomimetic activity has also been demonstrated in blood vessels, where tyramine, for example, releases preloaded tritiated noradrenaline from cat cerebral and femoral arteries (Marin & Recio, 1982).
Directly acting sympathomimetic amines, such as noradrenaline and isoprenaline, are potentiated by chronic treatment with reserpine, due to an upregulation of the adrenoceptors (Chess-Williams et al., 1987).

7. Non-typical mechanisms of action of sympathomimetic amines

In spite of the plethora of evidence cited above that sympathomimetic amines exert their effects via the release of endogenous noradrenaline, there are a number of observations which indicate that other mechanisms may also be involved. For such a diverse group of amines it is perhaps not surprising that other mechanisms, both direct and indirect, are involved in their pharmacological responses. It is, however, worth considering the various known mechanisms before we can identify new target receptors for trace amines. The suggestion that the pressor effects of tyramine may be mediated in part by a noradrenaline-independent mechanism comes from a dissociation between changes in blood pressure and plasma noradrenaline levels following injection of tyramine (Bianchetti et al., 1982; Chalon et al., 2002). Depletion of noradrenaline stores by reserpine was shown by Burn and Rand (1958) to only partially inhibit the pressor responses to tyramine in spinal cats. Reserpine pre-treatment has also been shown to not fully inhibit responses to tyramine in rabbit (Hudgins & Fleming, 1966) and rat (Maling et al., 1971) aorta and rat atria (Rice et al., 1987). The explanation for this lack of inhibition has been that the responses are direct (Maling et al., 1971; Krishnamurti & Grollman, 1972), possibly through α-adrenoceptors in the case of the aorta. We also showed that relaxation of rat uterus to tyramine was not affected by chronic reserpine treatment (Hawthorn & Broadley, 1984). We suggested that it was due to a non-sympathomimetic effect, “possibly upon receptors for phenylethylamines”. Cocaine, which inhibits the transport of sympathomimetic amines into sympathetic neurones, did not completely inhibit the contractions of rabbit aorta to tyramine (Furchgott et al., 1963). These authors also showed that residual contractile responses after reserpine treatment were not inhibited by cocaine and concluded that they were a direct effect of tyramine.

Tachyphylaxis is a characteristic feature of indirectly acting sympathomimetic amines in that repeated dosage results in a gradual decline of the response (Day, 1967; Liles et al., 2006) (Fig. 3). This is probably due to progressive exhaustion of stored noradrenaline, which is replaced in the vesicle by the less active amine. Responses are generally not completely abolished by tachyphylaxis, indicating that there is a residual response that is not due to release of noradrenaline. Also, there is a notable lack of cross-tachyphylaxis between certain amines, for example, development of tachyphylaxis to dexamphetamine in the rat or mephentermine in the cat is not crossed to tyramine (Day, 1967) (Fig. 3). Rapid tachyphylaxis of the pressor response to mephentermine (Fawaz & Simaan, 1965) or desamphetamine (Eble & Rudzik, 1965) in dogs was poorly crossed with tyramine. An explanation involving inhibition of MAO by the first administered amine, to leave the effects of tyramine potentiated, has been discounted since MAO inhibitors in fact enhance the tachyphylaxis to tyramine. Day (1967) suggested that this lack of cross-tachyphylaxis was due to amines having different mechanisms of release of noradrenaline. However, to date, no satisfactory explanation for these discrepancies has been put forward (Broadley, 1996).

Certain trace amines may exert direct actions on established receptors other than adrenoceptors. For example, tryptamine causes vasoconstriction in equine digital blood vessels, which was inhibited by the 5-HT receptor antagonist, ketanserin (Elliott et al., 2003). Tyramine (Jacob et al., 2003), methylphenidate and amphetamine (Iversen, 2006) are also capable of releasing dopamine.

We have shown that the amphetamines, cathinone, the active constituent of khat leaves, and 3,4-methylenedioxymethamphetamine (“ecstasy”, MDMA) (Al-Motarreb et al., 2002a; Al-Motarreb & Broadley, 2003; Baker et al., 2007) and (1'-PEA (Herbert et al., 2009) cause coronary vasoconstriction through a mechanism not involving α1-adrenoceptors.
and not dependent on release of noradrenaline following cocaine-sensitive neuronal uptake of these amines (Fig. 5). Furthermore, we have also demonstrated that the responses to ecstasy are not prevented by antagonists of angiotensin, endothelin, 5-hydroxytryptamine, prostaglandins or leukotrienes (Baker & Broadley, 2003). Thus, none of these vasoconstrictor autacoids or their receptors can explain the vasoconstriction. The possibility therefore arises that the vasoconstriction is due to an, as yet, unidentified receptor.

8. Trace amine receptors

The receptors through which trace amines exert these non-classical receptor-mediated responses may therefore be specific phenylethylamine receptors. The presence of binding sites for tryptamine, tyramine and β-phenylethylamine was muted many years ago (Ungar et al., 1978; Nguyen & Juorio, 1989). High affinity binding sites for [3H]-tryptamine (Kellar & Cascio, 1982; Perry, 1986; McCormack et al., 1986), [3H]-tyramine (Ungar et al., 1977; Vaccari, 1986) and [3H]-β-PEA (Hauger et al., 1982) were revealed in rodent brain. At about the same time, we showed that the relaxation response of the rat uterus to tyramine (Hawthorn & Broadley, 1984) and contraction of the guinea-pig trachea and lung (Hawthorn et al., 1985) were resistant to reserpine-treatment and proposed that these responses were mediated via a phenylethylamine receptor. It was not until 2001 that an extensive family of novel G-protein-coupled receptors, known as trace amine receptors (TAAR), was discovered.

The detection of the first trace amine receptor was made independently by two groups of investigators each using similar methods. Complex mixtures of oligonucleotides, whose sequences were based on serotonin (Borowsky et al., 2001) or dopamine (Bunzow et al., 2001) receptors, were used to amplify novel DNA sequences by PCR using rat cDNA and genomic DNA as templates (Zucchi et al., 2006). TAR1 represented one member of three related subfamilies of mammalian G-protein coupled receptors. Bunzow et al. (2001) cloned both rat and human TAR sequences and stably expressed them in human embryonic kidney (HEK) cells. Expression of the receptor in either Xenopus oocytes or HEK cells showed that exposure to β-PEA or tyramine led to the accumulation of cAMP (Zucchi et al., 2006). In contrast, classical biogenic amines like dopamine, noradrenaline, adrenaline, serotonin or histamine were not effective agonists for this receptor (Kim & von Zastrow, 2001; Zucchi et al., 2006). The TAR receptor family has been identified in human, chimpanzee, rat and mouse (Lindemann & Hoener, 2005; Lewin, 2006). In all species analyzed so far, three receptor subfamilies have been identified (Lindemann & Hoener, 2005). Because only subfamily 1 respond to trace amines (Borowsky et al., 2001), these receptors are now called trace amine-associated receptors (TAARs).

In this new nomenclature, TAR1 is now called TAAR1 and TAR2 is called TAAR4 (Lindemann & Hoener, 2005; Lewin, 2006).

So far, 19 individual TAARs for rat, 9 TAARs each for human and chimpanzee, and 16 for mouse have been identified (Lewin, 2006). A considerable homology had been reported between the human (hTAARs) and rat (rTAARs) TAARs (Lewin, 2006). In general, the potency of TAs, in particular β-PEA and tyramine, to activate TAAR4 in transfected cell lines is lower compared to their potency at TAAR1 (Borowsky et al., 2001; Davenport, 2003). The human and rat TAAR1 bind tyramine and β-PEA, while at the rat TAAR4, β-PEA is a very weak agonist for cAMP accumulation but tyramine is inactive (Borowsky et al., 2001). Tyramine is the most potent agonist known for human and rat TAAR1 expressed in COS-7 (African green monkey kidney fibroblast) and HEK293 cells (human embryonic kidney cell line). β-PEA is also an agonist for TAAR1 in transfected cells, but to a lesser degree (Borowsky et al., 2001; Lindemann & Hoener, 2005).

In transfected cell lines, the most commonly recognized signal transduction pathway for TAARs is stimulation of adenylate cyclase through the α subunit of stimulatory guanine nucleotide (Gs) protein to increase cAMP levels (Bunzow et al., 2001; Borowsky et al., 2001; Lewin, 2006). In intact tissue, tyramine produces vasorelaxation by reduction of inositol 1,4,5-trisphosphate (IP3) formation (Varma & Chemtob, 1993). Whether this response was mediated via TAAR was not determined.

The tissue localization of mRNA for the human TAAR1 has been detected by quantitative reverse transcription (RT)-PCR and shows low levels in discrete regions within the brain and in several peripheral tissues. Moderate levels were expressed in the stomach, kidney, and lung, while lower levels were found in the liver, prostate, skeletal muscle and spleen (Borowsky et al., 2001). In rat heart tissue, transcripts for at least 5 TAAR subtypes, including TAAR1, have been detected by RT-PCR (Chelliini et al., 2007) together with [3H]-tyramine binding sites (Frascaredi et al., 2008). The response of rat isolated cardiac tissue to β-PEA is a negative inotropy, which is opposite to that of a sympathomimetic agent and this response has been attributed to stimulation of TAAR1 (Frascaredi et al., 2008). In rat vascular tissue, we have demonstrated the presence of TAAR1 receptor protein by Western blot analysis and mRNA by RT-PCR (Fehler et al., 2008b). The contractile responses of porcine isolated coronary artery rings (Herbert et al., 2009) and rat aortic rings (Fehler et al., 2008a) to β-PEA and tyramine are not antagonized by inhibitors of receptors known to induce vascular contraction, such as α1-adrenoceptors, histamine and 5-HT receptors. Therefore it is likely that these responses are mediated via trace amine-associated receptors. Further definitive studies to characterize the roles of TAAR in the functional responses to trace amines and amphetamines are hampered by the current lack of selective antagonists.

As described above, the second messenger pathway established for cloned TAA receptors expressed in cell lines is cAMP (Bunzow et al., 2001). However, whether the functional vasoconstrictor responses to trace amines are mediated via adenylyl cyclase and cAMP generation is most unlikely. The response of rat isolated cardiac tissue to β-PEA is a negative inotropy, which is not consistent with the increases in force of cardiac contraction associated with increases in cAMP that occur with β-adrenoceptor agonists or the direct activator of adenylyl cyclase, forskolin (Chilliini et al., 2007). The contractile responses to a range of trace amines and amphetamines that we have observed in rat, guinea-pig and porcine vascular preparations are also not consistent with an increase in cAMP as the transduction pathway. In the rat heart, it has been suggested that dephosphorylation of critical tyrosine residues, possibly by activation of phosphatases, may mediate the responses to TAAR1 stimulation. Alternatively, tyrosine kinases might be the targets of TAAR1-dependent tyrosine phosphatases. The negative inotropic response to β-PEA is a phenomenon that has been observed in many cell types and is not specific to TAAR1. The contractile responses to trace amines remains to be evaluated.

An important question is whether the levels of endogenous ligands reach sufficient concentrations to activate TAARs. Basal plasma concentrations of trace amines, such as tyramine, in humans are notoriously difficult to measure because of their poor stability; the half-life of tyramine is 0.533 h (Van den Berg et al., 2003). However, recent improvements in technology have shown by means of reverse phase high performance liquid chromatography, basal serum levels of tyramine of 350 ng/ml (2.6 µM) in males and 320 ng/ml (2.34 µM) in females (Mao et al., 2009). Therefore normal circulating levels of tyramine are not expected to activate TAAR1 in the functional responses to trace amines. However, recent improvements in technology have shown by means of reverse phase high performance liquid chromatography, basal serum levels of tyramine of 350 ng/ml (2.6 µM) in males and 320 ng/ml (2.34 µM) in females (Mao et al., 2009). Therefore normal circulating levels of tyramine are not expected to activate TAAR1 in the functional responses to trace amines. Basal plasma concentrations of trace amines, such as tyramine, in humans are notoriously difficult to measure because of their poor stability; the half-life of tyramine is 0.533 h (Van den Berg et al., 2003). However, recent improvements in technology have shown by means of reverse phase high performance liquid chromatography, basal serum levels of tyramine of 350 ng/ml (2.6 µM) in males and 320 ng/ml (2.34 µM) in females (Mao et al., 2009). Therefore normal circulating levels of tyramine are not expected to activate TAAR1 in the functional responses to trace amines. However, recent improvements in technology have shown by means of reverse phase high performance liquid chromatography, basal serum levels of tyramine of 350 ng/ml (2.6 µM) in males and 320 ng/ml (2.34 µM) in females (Mao et al., 2009). Therefore normal circulating levels of tyramine are not expected to activate TAAR1 in the functional responses to trace amines.
However, in isolated tissues and in vivo, the effects of uptake into neurons and platelets, diffusion barriers and metabolism by MAO will mean that significantly higher concentrations would need to be used to achieve these EC50 concentrations at the receptor. Furthermore, EC50 values derived from binding data or generation of cAMP in cell lines would be several-fold less than those determined for a functional tissue response because of the signal amplification in coupling between receptor activation and the contractile response. Thus, normal circulating levels of tyramine and β-phenylethylamine would appear to be within the required range to elicit responses via TAARs.

 Plasma levels of tyramine and β-PEA can, however, be elevated after meals rich in these amines and in individuals in who MAO A and B activity is compromised or blocked. Plasma levels of tyramine are also elevated in hypertensive patients (Andrew et al., 1993) and in patients with migraine headaches (D’Andrea et al., 2004). Pressor responses are typically associated with a high dietary intake of tyramine, the oral dose of which to produce a 30 mm Hg rise in systolic blood pressure is approximately 7 mg/kg body weight (Peatfield et al., 1983). Much lower levels will be required in individuals taking MAO inhibitors (Zucchi et al., 2006), in smokers who have lower MAO A and B levels (Berlin & Anthenelli, 2001) and in women taking isoflavones as an alternative to hormone replacement therapy (Hutchins et al., 2005) and readily achievable with tyramine-rich fermented foods. Levels of cathinone (83 ng/ml) (Halket et al., 1995) and MDMA (180 ng/ml) (Samyn et al., 2002) detected in the plasma are also capable of stimulating TAARs. The concentrations of trace amines and amphetamines required to induce constrictions of isolated blood vessels in our studies are in the range 10–100 μM (Herbert et al., 2008). These concentrations are therefore compatible with the reported physiological levels required to activate TAAR1 expressed in cell lines and expected to be found in the circulation.

 A further potential endogenous ligand for TAARs is 3-iodothyronamine, an endogenous metabolite produced by thyroid hormone decarboxylation (Scanlan et al., 2004). 3-Iodothyronamine has been detected in the blood of humans, rats and mice and nanomolar concentrations can activate TAAR1. 3-Iodothyronamine, like the trace amines, causes negative inotropy and it has been proposed that thyroid hormone may have a role in control of cardiac function through this metabolite (Chielini et al., 2007). It is of interest that thyronamine is also a decarboxylated and deiodinated derivative of thyroxine and was shown over 30 years ago to exert positive rather than negative inotropic effects (Côté et al., 1974). It was classed as a sympathomimetic agent and had selective effects on the force of contraction and chronotropic actions were not entirely due to β-adrenoceptor stimulation (Côté et al., 1974) and the authors concluded that “there is a small [positive] inotropic effect which persists in the presence of alpha- and beta-adrenergic blockade, and is presumably due to a direct effect of the drug”.

9. Clinical implications of the vascular effects of trace amines and amphetamines

 A knowledge of the mechanisms whereby dietary trace amines and amphetamines exert their cardiovascular effects is essential if their undesirable haemodynamic effects are to be counteracted or prevented by appropriate drug therapy. Undesirable effects are usually encountered when excessive levels occur in the circulation through increased intake. There are reports of elevated levels of trace amines in various clinical disorders, including patients with primary headaches (D’Andrea et al., 2004). The question arises as to whether foods containing trace amines such as chocolate can trigger migraine headache, possibly through their vasoconstrictor actions. There are reports in the literature that have shown a relationship between tyramine ingestion and migraine headache in dietary migraineurs (Hanington, 1968; Millichap & Yee, 2003). One ingredient of chocolate that is frequently considered as the culprit for triggering migraine headaches is β-PEA, possibly through reduced cerebral blood flow by release of noradrenaline from sympathetic nerves (Millichap and Yee, 2003). A double-blind controlled study of adult migraineurs who believed that their headache was provoked by chocolate showed that in 5/12 subjects, chocolate ingestion was followed by a typical headache, whereas placebo had no effect (Gibb et al., 1991). In contrast, two other controlled trials have failed to confirm chocolate as a migraine trigger (Moffett et al., 1974; Marcus et al., 1997). In a survey of the literature, it was concluded that there was no evidence of a relationship between oral biogenic amines and food intolerance reactions, including migraine (Jansen et al., 2003). It is possible that there exist a population of migraine patients who are sensitive to tyramine in chocolate, possible due to reduced MAO activity (Millichap & Yee, 2003). Dietary trace amines may also have a role in hypertension since there is a suggestion that elevated plasma levels of tyramine may occur in hypertensive patients (Andrew et al., 1993). Furthermore, the rise in systolic blood pressure in response to tyramine infusion was significantly greater in hypertensive individuals than in normotensives (Colombo et al., 1989). One interpretation of this result is that hypertensives are more sensitive to the vasoconstrictor actions of tyramine mediated via trace amine-associated receptors. The discrepancy between levels of noradrenaline released into the plasma and the pressor response to tyramine led to the suggestion that the pressor response to tyramine may be mediated in part by a noradrenaline-independent mechanism (Blanchetti et al., 1982; Chalon et al., 2002). This other mechanism could be explained by a vasoconstriction through vascular trace amine-associated receptors. Tachyphylaxis of the pressor responses to trace amines and amphetamines has been well documented in animals (Day, 1967). However, there is less information about this phenomenon in humans. There does not appear to be tolerance to the pressor effects of tyramine (Cantarini et al., 2004) or phenylpropanolamine (O’Connell & Gross, 1991) or of the decongestant properties of phenylpropanolamine (Holmberg & Bende, 1988). It is worth remembering that the decongestant properties of amphetamines and their analogues are due to vasoconstriction in the nasal mucosa, which are presumed to arise from sympathomimetic effects (Broadley, 1996). In the light of the above discussions, it is possible that TAAR mechanisms may be involved but as yet these have not been studied.

Finally, of clinical relevance is the myocardial toxicity of the excessive social use of amphetamines, including cathinone and ecstasy. Since the late 1980s, increasing numbers of reports have detailed adverse reactions to ecstasy abuse (Davison & Parrott, 1997), which in some cases have proved fatal (Milroy et al., 1996). While hyperthermia is believed to be a major factor leading to death by ecstasy poisoning (Henry et al., 1992; Coore, 1996), post-mortem studies suggest hyponatraemia and myocardial fibrosis also as causes of death (Milroy et al., 1996)). Ecstasy use has been associated with cardiovascular collapse and sudden death (Dowling et al., 1987; Bedford Russell et al., 1992; Milroy et al., 1996), ventricular tachycardia and hypertension (Mas et al., 1999; Lester et al., 2000). In a survey of 11,011 cases of acute myocardial infarction (AMI), amphetamine abuse was significantly associated with AMI (Westover et al., 2008). Studies in The Yemen have shown a strong link between khat chewing and the onset of AMI (Al-Motarreb et al., 2002b; Al-Habori, 2005). Furthermore, a case–control study has shown that khat chewing is an independent dose-related risk factor for the development of AMI and it was suggested that the cathinone content of khat was responsible for precipitating the AMI (Al-Motarreb et al., 2005). Normal doses of pseudoephedrine (Pederson et al., 2001) and phenylpropanolamine (Pilszek et al., 2003) have also been associated with acute myocardial ischaemia and infarction.

Further examples of cardiovascular toxicity arising from abuse of amphetamines include reports of pulmonary hypertension with methamphetamine (Thompson, 2008) and intracerebral haemorrhage.
with MDMA (Harries & De Silva, 1992; Gledhill et al., 1993). More than 30 case reports have described intracerebral or subarachnoid haemorrhage following either recommended or excessive doses of phenylpropanolamine, which are attributed to hypertension or cerebral vasospasm (Kernan et al., 2000). A multicenter case–control study confirmed phenylpropanolamine as an independent risk factor for haemorrhagic stroke with an odds ratio of 16.58 for women using appetite suppressants and 3.13 for women using cough or cold remedies containing phenylpropanolamine. It was concluded on the basis of this study that phenylpropanolamine causes between 200 and 400 strokes annually in the United States (Brust, 2003). In 2001, the FDA ordered products containing phenylpropanolamine to be withdrawn from the market (Mersfelder, 2001). Ischaemic and haemorrhagic strokes have also been reported in ephedrine users (Wooten et al., 1983) and recreational users of dietary supplements containing ephedra alkaloids (“ma huang”) (Haller & Benowitz, 2000), and intracranial haemorrhage has followed pseudoephedrine use (Loizou et al., 1982). Their over-the-counter availability however continues.

In view of the vasoconstrictor actions of these trace amines and amphetamines on the coronary circulation discussed above, it is likely that the myocardial ischaemia and infarction that has occurred following their use and abuse is due to restriction of coronary flow to the heart. If TAARs mediate a substantial part of this response then these receptors may play a significant role in the cardiac toxicity to these amines. A need for a selective antagonist for TAARs is therefore indicated as a potential means of treating and preventing these cardiac consequences of amphetamine abuse. Although a role for TAARs in the cerebral circulation is still not known, the same arguments may apply to ischaemic and haemorrhagic strokes arising from phenylpropanolamine use.

10. Conclusions

Sympathomimetic amines, trace amines, amphetamines, dietary amines—these terms are often used interchangeably for a group of compounds yielding common pharmacological responses. On the vasculature, these responses are usually vasoconstriction, leading to increases in blood pressure. We have seen, however, that the mechanism for the increase in blood pressure and vasoconstriction is not straightforward and our knowledge on their actions has evolved over the past century. It has become clear that there are differences between species and between different amines. For example, in humans the increase in blood pressure with tyramine is due mainly to increased cardiac output, although vasoconstriction no doubt occurs in selected vascular beds. The pharmacological mechanism for the vasoconstriction does not appear to be due solely to indirect sympathomimetic actions and release of noradrenaline by the amines. Most recent studies show that trace amines and amphetamines can interact with trace amine-associated receptors which are located in blood vessels and the vasculature may be due to activation of these receptors. Whether this mechanism of action can explain many of the pharmacological effects of trace amines and the cardiovascular toxicity of amphetamines remains to be established. Also, whether the lack of cross-tachyphylaxis of the pressor responses between different amines can be explained by their actions on trace amine-associated receptors remains to be established. It is worthy of note that the persistent pressor response to tyramine in rats after dexamphetamine tachyphylaxis is unlikely to be due to TAAR activation as rat blood vessels are notably insensitive to tyramine, where it behaves as a partial agonist (Feather et al., 2008a). It appears that for a group of drugs whose cardiovascular pharmacology was first described in detail a century ago (Barger & Dale, 1910), there still remains much to be learnt about their vascular actions. Perhaps it is also time to review the nomenclature of this group of amines. Since they are not all trace amines (i.e. found endogenously in trace amounts) and their actions are not always due to indirect sympathomimetic activity, these would seem inappropriate terms. Perhaps the term phenylethylamine would be more a accurate description (although that would discount tryptamine!) and the response to these amines “phenylethylaminergic.”

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