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The influence of structure on the accumulation of caffeine induced by methyl xanthine derivatives

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Abstract—In rats given caffeine (25 mg kg^{-1} p.o.) and 1,3,8-trisubstituted xanthine (1,3,8-TSX) derivatives (10 mg kg^{-1} p.o.) the accumulation of the former in plasma was 300% higher than that in control animals given caffeine alone. The effect on caffeine accumulation appears to be independant of the nature of the N^3 substituent and its absence in rats given 1,3-disubstituted xanthines (1,3-DSX) instead of 1,3,8-TSX suggests that the presence of the C^8-methyl group in the latter compounds is responsible for the accumulation phenomenon. The results of our previous work imply that these observations in this rat model can be extrapolated to man.

It has been recently reported (Segura & Tarrús 1984; Tarrús et al 1987a) that the new xanthine derivative, furafylline (1,8-dimethyl-3-(2′-furfuryl)-methyl-xanthine inhibits the metabolism of caffeine (1,3,7-trimethyl-xanthine) in man leading to the appearance of adverse effects related to caffeine accumulation. The habitual consumption of caffeine containing foods and beverages is extensive worldwide and the detection of interactions similar to that described for furafylline is of obvious interest. The finding that N^3-demethylation, a common metabolic pathway of caffeine in both rat and man (Cornish & Christman 1957; Khanna et al 1972; Welch et al 1977; Wietholtz et al 1981; Tarka 1982; Tarrús et al 1987b), is inhibited by furafylline in both species has permitted the development of a suitable animal model to detect the phenomenon (Tarrús et al 1987b) and in the present work this model has been used to study the possible relationship between xanthine structure and caffeine accumulation in the blood.

Material and methods

The trimethylxanthines (TX) and dimethylxanthines (DX) were synthesized at Laboratorios Almirall, S.A., Barcelona, Spain. Caffeine and theophylline were purchased from Merck, Darmstadt, West Germany, as were chloroform, methanol, acetonitrile, sodium acetate, *N,N*-dimethylacetamide and acetic acid. Sodium hyroxide and hydrochloric acid were purchased from Panreac, Barcelona, Spain. Other compounds used were Viscontran L-52 (cellulose methylether from Henkel, Barcelona, Spain), 5% heparin solution (Laboratorios Leo, Madrid, Spain) and pure nitrogen (Carburos Metálicos, Barcelona, Spain). Deionized and twice distilled water was used throughout the study.

Male Wistar rats (200–250 g) were fasted overnight before the oral administration of the test compounds (Table 1). The schedule of drug administration and blood sampling is shown in Figs 1, 2. Caffeine (0·025% w/v) and theophylline (0·066% w/v) were given as aqueous solutions and all other compounds as 0·066% w/v suspensions prepared as follows:

The drug was suspended in water and sodium hydroxide (1 M) was added dropwise to give a clear solution (ca pH 12). Viscontran L-52 (final concentration 20 mg mL^{-1}) was added and the mixture degassed under vacuum until totally transparent. Dilute hydrochloric acid was added dropwise with mild shaking to obtain a final pH of 6·5–7·5. The resulting suspension was made up to the desired volume with water. These doses and schedules were chosen from previous experimental results

Table 1. Structure of the trisubstituted and disubstituted xanthines tested in this study.

	Substituents[a]			
Compounds	R_1	R_3	R_7	R_8
Caffeine	Methyl	Methyl	Methyl	H
A	Methyl	2-Thenyl	H	Methyl
B	Methyl	Benzyl	H	Methyl
C	Methyl	3-Cyclohexenyl-1-methyl	H	Methyl
D (D-4026)	Methyl	Phenyl	H	Methyl
E (Furafylline)	Methyl	2-Furfuryl	H	Methyl
F	Methyl	Methyl	H	Methyl
G (Verofylline)	Methyl	2-iso Pentyl	H	Methyl
H (Theophylline)	Methyl	Methyl	H	H
I	Methyl	2-Furfuryl	H	H
J	2-Furfuryl	2-Furfuryl	H	H
K	2-Furfuryl	Methyl	H	H
L	n-Propyl	2-Furfuryl	H	H
M	Methyl	2-(1,3-Dioxo-lanyl) Propyl	H	H

(a) The numbering refers to the positions of the substituents in the xanthine (3,7-dihydro-1H-purine-2,6-dione) nucleus.

Table 2. Chromatographic conditions used to separate and quantitate the drugs studied. All eluents were adjusted to pH 4·0 with acetic acid. The wavelength of detection was 280 nm.

Eluent[a]	Flow rate (mL min^{-1})	Substance	Retention Time (min)	Relative[b] Retention (caffeine = 1·0)
NaAc/MeOH (2:3)	1·4	Caffeine	3·4	1·0
		A	7·4	3·9
		B	9·0	5·0
		C	12·4	7·4
NaAc/MeOH (1:1)	1·4	Caffeine	3·8	1·0
		J	8·4	3·6
		L	9·4	4·1
		G	14·6	7·0
NaAc/MeOH (4:3)	1·4	Caffeine	4·5	1·0
		D	7·2	2·1
NaAc/MeOH (3:2)	1·4	Caffeine	5·0	1·0
		E	6·0	1·3
NaAc/Acn (8:2)	2·4	F	2·6	0·6
		Caffeine	3·6	1·0
NaAc/MeOH (8:1·5)	2·0	Caffeine	5·9	1·0
		M	7·6	1·4
NaAc/Acn (8·6:1·4)	2·0	H	3·4	0·4
		Caffeine	6·1	1·0
		I	11·2	2·1
		K	11·2	2·1

(a) NaAc: 0·02 M Sodium Acetate; MeOH: Methanol, Acn: Acetonitrile.
(b) Taking into account the void volume.

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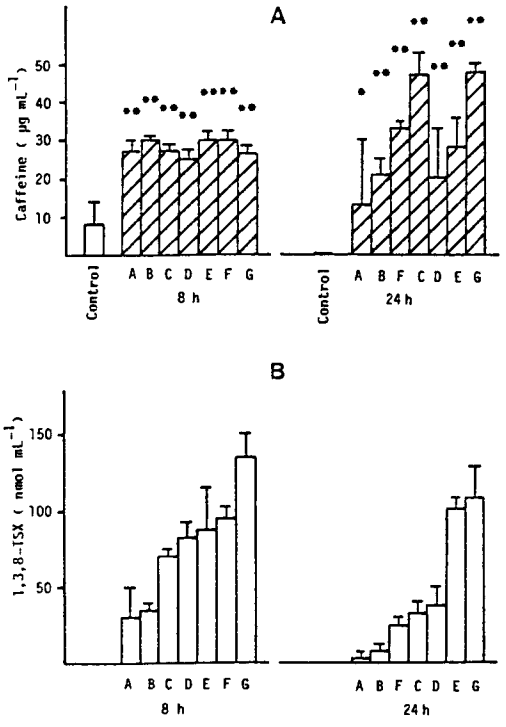

FIG. 1. (A) Plasma levels of caffeine (μg mL^{-1}) in rats given 10 mg kg^{-1} of 1,3,8-trisubstituted xanthines (1,3,8-TSX) at 0 (0900 h), 4 and 8 h together with 25 mg kg^{-1} caffeine at 2 and 8 h (hatched bars, test group) or only 25 mg kg^{-1} caffeine at 2 and 8 h (open bars, control group). Each reported value is the mean $\pm$ standard deviation of 8 (control group) or 4 (test group) animals. The asterisks indicate a significant difference (*$P < 0{\cdot}0005$; ** $P < 0{\cdot}001$) between the control and the test groups. The structures of compounds A, B, C, D, E, F and G are described in Table 1. (B) Plasma levels of 1,3,8-TSX (nmol mL^{-1}) in the test group. No levels were detected in the control groups.

The levels of 1,3,8-TSX are expressed in nmol mL^{-1} in order to compare 1,3,8-TSX plasma levels against caffeine accumulation effect independently of the dose and molecular weight of the compound.

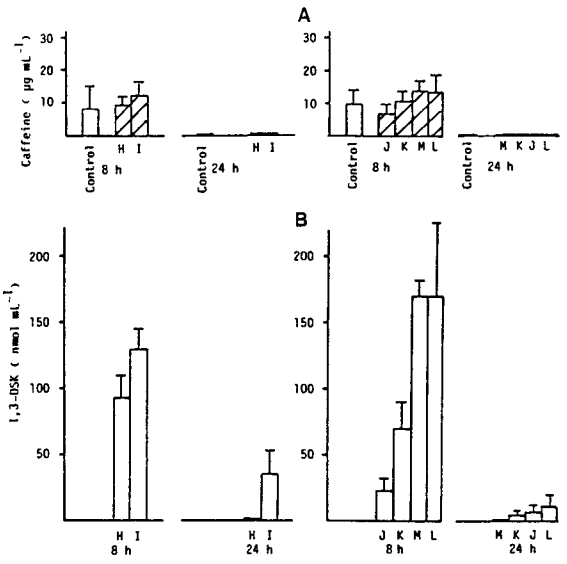

FIG. 2. (A) Plasma levels of caffeine in rats given 10 mg kg^{-1} of 1,3-disubstituted xanthines (1,3-DSX) at 0 (0900 h), 4 and 8 h together with 25 mg kg^{-1} caffeine at 2 and 8 h (hatched bars, test groups) or only 25 mg kg^{-1} caffeine at 2 and 8 h (open bars; control group). Each reported value is the mean $\pm$ standard deviation of 8 (control group) or 4 (test groups) animals. No significant differences ($P < 0{\cdot}01$) between the control and the test groups were detected. The structures of compounds H, I, J, K, L and M are described in Table 1. (B) Plasma levels of 1,3-DSX in the test groups. No levels were detected in the control groups.

The levels of 1,3-DSX are expressed in nmol mL^{-1} in order to compare 1,3-DSX plasma levels against caffeine accumulation effect independently of the dose and molecular weight of the compound.

(Tarrús et al 1987b) in order to obtain in rats the same accumulation effect on caffeine plasma levels as was shown in man (Tarrús et al 1987a).

Blood samples were collected into heparinized tubes via capillary tube punctures of the retro-orbital venous cavity under light ether anaesthesia and the plasma separated by centrifugation. The drugs were extracted from the plasma samples (1 mL) with chloroform (7 mL) mixing them by means of a tilt shaker (35 min). The organic layer was separated after centrifugation and evaporated to dryness under a stream of nitrogen. The residue was taken up in *N*,*N*-dimethylacetamide (100–200 μL) before being injected (50 μL) into a Waters high performance liquid chromotograph (HPLC) equipped with an injector (U6K), a solvent delivery system (M-6000A), an absorbance detector (440) operated at 280 nm and a reverse phase μ Bondapak C_{18} column preceded by an octadecilsilane filled guard column. The precise HPLC conditions for each group tested are reported in Table 2.

Statistical comparisons of caffeine plasma levels between test and control groups were made using Student's two-tailed *t*-test.

Results and discussion

Although both caffeine and furafylline are trisubstituted xanthine compounds and both bear methyl groups in the pyrimidine (position N^1) and imidazole rings (position N^7 or C^8), the size and polarity of the N^3-substituents are quite different. As a first approach we therefore studied the influence of varying the N^3-substituents in a series of 1,8-dimethyl xanthine derivatives (1,3,8-trisubstituted xanthines: 1,3,8-TSX; compounds A–G) on caffeine accumulation. In rats receiving caffeine only, the mean levels of caffeine reached were 7·83–9·99 μg mL^{-1} at 8 h and caffeine was undetectable at 24 h. The concurrent administration of 1,3,8-TSX compounds gave rise to a clear accumulation of caffeine (Fig. 1). Although the concentrations of the 1,3,8-TSX compounds ranged from 29·8 to 134·6 nmol mL^{-1} they caused a similar degree of caffeine accumulation at 8 h (24·8–30·7 μg mL^{-1}) suggesting saturation of the process. The caffeine levels found at 24 h (12·8–47·6 μg mL^{-1}) were less homogeneous probably due to variable plasma concentrations of 1,3,8-TSX (variable elimination half-lives) and/or different potencies on the accumulation effect at lower non-saturating concentrations. Nevertheless, it is clear that all 1,8-dimethyl-3-substituted compounds tested induced caffeine accumulation and the interaction appears to be independent of the alkyl or aromatic nature of the N^3-substituent.

By contrast, the only structural difference between the very potent compound F and caffeine is the position of the methyl group in the imidazole ring (C^8 and N^7, respectively). This suggests that the C^8-methylation is responsible for the effects on caffeine accumulation and certainly no such effects have been described for theophylline (compound F without C^8-methyl) despite its extensive clinical use for several decades. In accordance with this suggestion, when compounds H (theophylline) and I (furafylline without C^8-methyl) were tested (Fig. 1) they failed to produce any significant caffeine accumulation as compared to controls, even at high plasma concentrations (92·2–130·8 nmol mL^{-1}). Confirmation that other types of 1,3-disubstituted xanthines (1,3-DSX) also fail to cause caffeine accumulation was obtained by studying compounds J (N^1-aryl, N^3-aryl), L (N^1-alkyl, N^3-aryl) and M (N^1-alkyl, N^3-alkyl) (Fig.

2). Since some of these compounds had lipophilic properties similar to those of 1,3,8-TSX, as can be deduced from their reverse phase chromatographic retention times (Tables 2 and Christensen & Whitsett 1976), the accumulation of caffeine by the 1,3,8-TSX appears to be a function of their trisubstitution rather than of their overall lipophilic characteristics.

In conclusion, and based upon previously experimental observation (Tarrús et al 1987a,b), the results presented in this work indicate that compounds with a methyl group in position C^8, in addition to both N^1-methyl and N^3-aryl or alkyl substitution of the xanthine nucleus, are able to produce an accumulation of concurrently administered caffeine in blood, probably due to an inhibition of its metabolism. Our results also suggest that 1,3-disubstituted xanthines do not affect the metabolism of concurrently administered caffeine.

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Interaction between the cardiovascular effects of clonidine and the κ-opioid agonist U-50,488H in the anterior hypothalamic area of the rat brain

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Abstract—In thiobutabarbitone-anaesthetized rats, microinjection of clonidine (1–40 nmol) into the anterior hypothalamic area (AHy) produced dose-dependent reductions in mean arterial blood pressure and heart rate. Microinjection of the κ-opioid agonist U-50,488H (3 and 10 nmol) did not modify these parameters. Simultaneous co-administration of clonidine (4 nmol) and U-50,488H (10 nmol) into the AHy resulted in significant potentiation of the clonidine-induced hypotension and marked attenuation of the bradycardia. A lower dose of U-50,488H (3 nmol) co-administered with clonidine (4 nmol) did not influence the cardiovascular responses to clonidine. These findings suggest that AHy neurons involved in the cardiovascular responses to clonidine may be modulated by κ-opioid receptor stimulation.

The anterior hypothalamic area (AHy) of the rat brain is a site of central cardiovascular regulation (see Brody et al 1980; Abboud 1984) functioning as an integrative centre (Ciriello et al 1983) and as a modulatory influence on the baroreceptor reflex (Miyajima & Bunag 1985). Adrenoceptors of the α_2-subtype are present in the AHy (Young & Kuhar 1980; Unnerstall et al 1984) and their stimulation by direct microinjection of the imidazolidine derivative clonidine results in hypotension and bradycardia (Struyker-Boudier et al 1974). Similarly, opioid receptors of the κ subtype have been detected in the AHy (Lynch et al 1985) whilst the presence of the putative endogenous κ receptor agonist dynorphin (Corbett et al 1982) has been demonstrated immunohistochemically in this brain region (Khachaturian et al 1982). However, intrahypothalamic injection of κ-agonists in conscious rats produced no detectable cardiovascular effects (Pfeiffer et al 1982). On the other hand, McWilliam & Campbell (1987) have demonstrated that α_2-adrenoceptors and κ opioid receptors may interact in rat hypothalamic synaptosomes to modulate the release of noradrenaline.

In the present study we have examined the cardiovascular effects of co-administration into the AHy of the selective κ agonist U-50,488H (*trans*-3,4-dichloro-*N*-methyl-*N*-[2-(1-pyrrolidinyl) cyclohexyl] benzeocetamide) (Vonvoigtlander et al 1983) and clonidine in anaesthetized rats.

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Materials and methods

Male Wistar-Kyoto rats weighing between 250–350 g were anaesthetized with thiobutabarbitone sodium (Inactin, 100 mg kg^{-1}, intraperitoneally). The rats were tracheotomized and a polyethylene catheter (SP 45) was inserted into the right carotid artery for blood pressure and heart rate (HR) measurements. The arterial catheter was connected to a Gould-Statham pressure transducer which was coupled to a Grass polygraph recorder. HR was derived from the pressure signal using a tachometer (Grass, model 7P44B). The rats were then placed into a stereotaxic apparatus (David Kopf) and stainless steel needles (300 μm O.D.) were lowered bilaterally through cranial burr holes into the AHy. Stereotaxic co-ordinates were: A.P. −1·1 to −1·6 mm from bregma; ±0·5 mm lateral to midline; 9·0 mm ventral to the skull surface, in the flat skull position (Paxinos