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Caffeine induces central cholinergic analgesia

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Abstract The antinociceptive effect of caffeine was examined by using the hot-plate, abdominal constriction tests in mice and the tail flick and paw-pressure tests in rats. Caffeine (1–5 mg kg^{-1} s.c. in mice; 2.5–5 mg kg^{-1} i.p. in rats) produced significant antinociception in both species which was prevented by atropine (5 mg kg^{-1} i.p.), pirenzepine (0.1 μg per mouse i.c.v.), hemicholinium-3 (1 μg/mouse i.c.v.) and N^6-cyclopentyladenosine (5 μg/mouse i.c.v.), but not by naloxone (1 mg kg^{-1} i.p.), CGP 35348 (100 mg kg^{-1} i.p.), α-methyl p-tyrosine (100 mg kg^{-1} i.p.) and reserpine (2 mg kg^{-1} i.p.). Intracerebroventricular injection of caffeine in mice at doses (2.5–5 μg per mouse) which were largely ineffective by parenteral routes, induces an antinociception whose intensity equalled that obtainable s.c. or i.p. In the antinociceptive dose-range, caffeine did not produce any behavioural impairment as revealed by the rotarod and Irwing tests. On the basis of the above data, it can be postulated that caffeine exerts an antinociceptive effect mediated by central amplification of cholinergic transmission.

Key words Caffeine · Antinociception · Analgesia · Cholinergic system · Acetylcholine · Muscarinic receptors · Heteroreceptors · A_1-A_2 receptors

Introduction

Caffeine is the most popular psychoactive drug in the world (Max 1986). Many people who ingest caffeine experience various degrees of arousal characterized by reduced drowsiness and fatigue (Curatolo and Robertson 1983). Caffeine is used as an adjuvant analgesic for various types of pain such as headache, postpartum pain, postoperative pain, dental surgery pain (Sawynok and Yaksh 1993), in combination with non-steroidal anti-inflammatory drugs (Cass and Frederik 1962; Vinegard et al. 1976; Jain et al. 1978; Laska et al. 1984; Forbes et al. 1991; Schachtel et al. 1991; Ward et al. 1991; Sawynok and Yaksh 1993) and in combination with morphine (Person et al. 1985; Mirsa et al. 1985; Malec and Michalska 1988). In animal studies caffeine also has intrinsic antinociceptive properties in threshold tests (Person et al. 1985; Malec and Michalska 1988; Sawynok and Reid 1996a) and in inflammatory tests (Siegers 1973; Seegers et al. 1981; Sawynok and Reid 1995; Sawynok et al. 1995).

Caffeine (50 μM) produced an enhancement of acetylcholine (ACh) release in electrically stimulated rat brain slices (Pedata et al. 1984) and, at 100 μM, in rat hippocampal synaptosomes (Pedata et al. 1986). More recently, Carter et al. (1995) showed that the oral administration of caffeine dose-dependently increased the extracellular levels of ACh, through a selective antagonism of A_1 receptors, measured by microdialysis technique in the hippocampus of freely moving rats. Furthermore, in the rat cerebral cortex, caffeine reduced the inhibition of ACh release produced by morphine (Jhamandas et al. 1978; Phillis et al. 1980).

It has long been known that ACh (Pedigo et al. 1975), selective M_1 agonists such as McN-A-343 and AF-102B (Bartolini et al. 1992), unselective muscarinic agonists such as tremorine (Lenke 1958), oxotremorine (George et al. 1962; Bartolini et al. 1987), arecoline (Herz 1962), pilocarpine (Hendershot and Forsaith 1959) and cholinesterase inhibitors such as physostigmine (Harris et al. 1969; Ireson 1970) and diisopropyl phosphorofluoridate (Lentz et al. 1969) induce antinociception in laboratory animals by the activation of the cholinergic system. Moreover, the amplification of cholinergic neurotransmission induced by antagonism of muscarinic autoreceptors (Bartolini et al. 1989; Gualtieri et al. 1989; Ghelardini et al. 1990) or, alternatively, by interaction with heteroreceptors (Ghelardini et al. 1992) located on presynaptic cholinergic terminals, produces a central antinociceptive effect.

Since A_1 receptors are heteroreceptors of central cholinergic neurones which exert an inhibitory control of ACh release (Spignoli et al. 1984; Jackisch et al. 1984; Carter

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et al. 1995), we decided to investigate the involvement of the cholinergic system in the mechanism of analgesic action of caffeine in mice and rats.

Methods

Animals. Male Swiss albino mice (23–30 g) and Wistar rats (200–300 g) from the Morini breeding farm (San Polo d'Enza, Italy) were used. Fifteen mice and four rats were housed per cage. The cages were placed in the experimental room 24 h before the test for acclimatisation. The animals were kept at 23 ± 1° C with a 12 h light/dark cicle, light at 7 a.m., with food and water ad libitum.

Drugs. The following drugs were used: caffeine, atropine sulphate, α-methyl p-tyrosine methyl ester hydrochloride and physostigmine sulphate (Sigma, USA); hemicholinium-3 hydrobromide (HC-3); pirenzepine dihydrochloride, naloxone hydrochloride and N^6-cyclopentyladenosine (RBI); CGP 35348 (3-aminopropyl-diethoxymethyl-phosphinic acid), ascorbic acid and reserpine (Ciba Geigy, Switzerland); oxotremorine hydrochloride (Fluka); sodium chloride and acetic acid glacial (Merck).

Experimental design. Animals were randomly assigned to a control (saline solution) or a treated group (caffeine). Both groups received a pretreatment consisting of the injection of one of the following drugs: atropine, pirenzepine, naloxone, hemicholinium-3 (HC-3), CGP-35348, α-methyl p-tyrosine methyl ester, N^6-cyclopentyladenosine or reserpine. All the drugs were injected 15 min before treatment with the exception of HC-3, CGP 35348, α-methyl p-tyrosine and reserpine. HC-3, α-methyl p-tyrosine and CGP-35348 were administered respectively 5 h, 2 h and 5 min before treatment whereas reserpine was injected twice, 48 and 24 h, before the test. All animals used were drug naive. All experiments were carried out according to the Principles of Laboratory Animal Care and the guidelines of the European Community Council. All drugs were dissolved, immediately before use, in isotonic (NaCl 0.9%) saline solution except reserpine which was dissolved respectively in a 20% solution of ascorbic acid. Drug concentrations were prepared in such a way that they could be administered in a volume of 10 ml kg^{-1} subcutaneously (s.c.), intraperitoneally (i.p.) and of 5 μl/mouse or 10 μl/rat intracerebroventricularly (i.c.v.).

I.c.v. administration was performed under ether anaesthesia using isotonic saline as solvent, according to the method described for mice by Haley and McCormick (1957) and extrapolated to rats by us. Substances were injected at the necessary dose dissolved in 5 μl isotonic saline solution for mice and 10 μl for rats. To ascertain the exact drug injection point, some mice or rats were injected i.c.v. with 5 or 10 μl of diluted 1:10 Indian ink and their brains were examined macroscopically after sectioning.

Hot plate test. The method adopted was described by O'Callaghan and Holtzman (1976). Mice were placed inside a stainless steel container, thermostatically set at 52.5 ± 0.1° C in a precision waterbath from KW Mechanical Workshop, Siena, Italy. Reaction times (s), were measured with a stop-watch about 30 min before pretreatment (pretest) and 15, 30, 45, 60 and 90 min after treatment. The endpoint used was the licking of the fore or hind paws. Those mice scoring below 12 and over 18 s in the pretest were rejected (30%). An arbitrary cut-off time of 45 s was adopted at which the animals were removed from the hot-plate and given a score of 45 s.

Abdominal constriction test. The test was performed in mice according to Koster et al. (1959). Mice were injected i.p. with a 0.6% solution of acetic acid (10 ml kg^{-1}). The number of stretching movements was counted for 10 min, starting 5 min after acetic acid injection. Caffeine was administered 5 min before acetic acid injection.

Paw pressure. The nociceptive threshold in the rat was determined with an analgesimeter (Ugo Basile, Varese, Italy), according to the method described by Leighton et al. (1988). Threshold pressure was measured about 30 min before treatment (pretest) and 15, 30 and 45 min after treatment. Rats scoring below 50 g or over 85 g during the pretest were rejected (25%). An arbitrary cut-off value of 250 g was adopted.

Tail flick. An analgesimeter from Ugo Basile (Varese, Italy) was used to perform the tail- flick test described by D'Amour and Smith (1941). The light from a project bulb situated beneath the platform where the animal was placed, was focused through a small hole on the ventral part of the tail at a point about 4 cm from the tip. Withdrawal of the tail exposed a photocell to the light, which turned off the thermal stimulus and automatically stopped the clock. The intensity was regulated so that the reaction time varied between 2 and 4 s. The analgesia was tested before and 15, 30 and 45 min after treatment. Each value was derived from the mean of three consecutive readings in which the light was focused on three adjacent points of the tail.

Rota-rod test. The apparatus consisted of a base platform and a rotating rod of 3 cm diameter with a non-slippery surface. This rod was placed at height of 15 cm from the base. The rod, 30 cm in length, was divided into 5 equal sections by 6 disks. Thus up to 5 mice were tested simultaneously on the apparatus, with a rod-rotating speed of 16 rpm. The integrity of motor coordination was assessed on the basis of endurance time of the animals on the rotating rod. One day before the test, the animals were trained twice. On the day of the test only the mice that were able to stay balanced on the rotating rod between 70 and 120 s (cut-off time) were selected for testing. The performance time was measured before and at various times after treatment.

Irwing test. The test was performed according to the method described by Irwing (1966).

Statistical analysis. Results are given as the mean ± S.E.M.; analysis of variance, followed by Scheffe's *F* procedure for post-hoc comparison, was used to verify the significance between two means. *P* values of less than 0.05 were considered significant. Data were analysed with the StatView for Macintosh (1992) computer program.

Results

The antinociceptive effect of caffeine was investigated using the hot-plate and abdominal constriction tests in mice and the tail flick and paw pressure tests in rats.

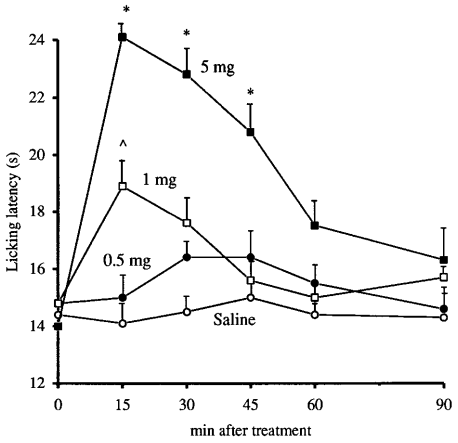

Fig. 1 Dose response curves of caffeine administered s.c. in the mouse hot-plate test. The doses are expressed as mg kg^{-1}. *Vertical lines* show SE mean. ^ $P < 0.05$; * $P < 0.01$ in comparison with saline controls. Each point represents the mean of at least 10 mice

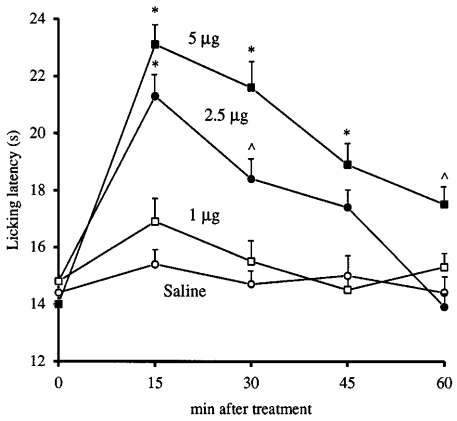

Fig. 2 Dose response curves of caffeine administered i.c.v. in the mouse hot-plate test. The doses are expressed as µg/mouse. *Vertical lines* show SE mean. $P < 0.05$; * $P < 0.01$ in comparison with saline controls. Each point represents the mean of 10–12 mice

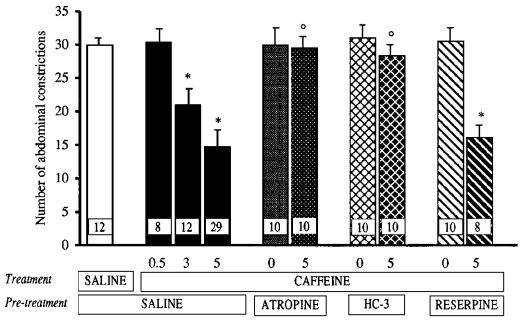

Fig. 3 Dose-response curve of caffeine and effect of atropine (5 mg kg^{-1} s.c.), hemi-cholinium-3 (HC-3) (1 µg/mouse i.c.v.) and reserpine (2 mg kg^{-1} i.p.) pretreatments of antinociception induced by caffeine (5 mg kg^{-1} s.c.) in the mouse abdominal constriction test. Atropine and HC-3 were injected respectively 15 min and 5 h before caffeine treatment. Reserpine was administered twice 48 and 24 h before test. The nociceptive responses were recorded 10–20 min after caffeine administration. *Vertical lines* show SE mean. * $P < 0.01$ in comparison with saline controls; ° $P < 0.01$ in comparison with saline-caffeine treated mice. Numbers inside the columns indicate the number of mice

In the hot-plate test caffeine, injected s.c., in the range of doses of 1–5 mg kg^{-1} (Fig. 1), and i.c.v. (2.5–5 µg/mouse) (Fig. 2) induced a significant increase in the pain threshold. The antinociceptive effect reached a maximum 15 min after administration and then diminished, disappearing within 60 min. Table 1 shows that caffeine antinociception was completely prevented by the antimuscarinic drug pirenzepine (0.1 µg/mouse i.c.v.), the choline uptake blocker HC-3 (1 µg/mouse i.c.v.) and the A_1 agonist N^6-cyclopentyladenosine (5 µg/mouse i.c.v.). Conversely, no modification in caffeine antinociception was obtained by pretreating mice with the opioid antagonist naloxone (1 mg kg^{-1} i.p.), the $GABA_B$ antagonist CGP 35348 (100 mg kg^{-1} i.p.) and the monoamine synthesis inhibitor α-methyl p-tyrosine (100 mg kg^{-1} i.p.) (Table 1).

The dose-response curve of caffeine administered s.c. (3–5 mg kg^{-1}) in the abdominal constriction test is shown in Fig. 3. The reduction in the number of abdominal constrictions caused by caffeine (5 mg kg^{-1} s.c.) was prevented by pretreatment with the antimuscarinic agent atropine (5 mg kg^{-1} i.p.) and HC-3 (1 µg/mouse i.c.v.), but was not modified by reserpine (2 mg kg^{-1} i.p.), a monoamine store depletor (Fig. 3).

As shown in Table 2, caffeine antinociception was confirmed in the rat paw-pressure test. Caffeine, administered i.p. at the dose of 1–5 mg kg^{-1}, induced antinociception, peaking after 15 min and persisting up to 30 min (Table

Table 1 Effects of pirenzepine, hemicholinium-3 (HC-3), N_6-cyclopentyladenosine (N_6-CPA), naloxone, CGP-35348 and α-methyl-p-tyrosine (αMpT) on antinociception caused by caffeine in the mouse hot-plate test

Pretreatment	Treatment	mg s.c.	Licking latency (s)			
			Before pretreatment	After treatment		
				15 min	30 min	45 min
Saline 10 ml · kg^{-1} i.p.	Saline		13.5 ± 0.9	14.3 ± 1.1	15.2 ± 0.7	14.2 ± 0.9
Saline 5 µl/mouse i.c.v.	Saline		14.2 ± 0.8	15.1 ± 1.2	14.3 ± 1.0	15.3 ± 1.1
Saline	Caffeine	5	14.5 ± 0.9	25.4 ± 1.6*	22.3 ± 1.8*	19.4 ± 1.8**
Pirenzepine 0.1 µg/mouse i.c.v.	Saline		14.1 ± 1.1	15.7 ± 1.3	14.6 ± 1.8	15.2 ± 1.6
	Caffeine	5	15.1 ± 0.7	17.6 ± 1.5***	16.9 ± 2.0***	16.2 ± 1.5
HC-3 1 µg/mouse i.c.v.	Saline		13.7 ± 0.5	14.8 ± 1.2	13.8 ± 1.1	13.5 ± 1.5
	Caffeine	5	14.1 ± 0.8	17.8 ± 1.2***	16.7 ± 2.1***	16.5 ± 1.6
N^6-CPA 5 µg/mouse i.c.v.	Saline		13.9 ± 0.7	15.0 ± 0.9	15.7 ± 1.1	16.4 ± 1.7
	Caffeine	5	14.3 ± 0.6	16.1 ± 1.8***	16.5 ± 1.5***	15.5 ± 1.2
Naloxone 1 mg kg^{-1} i.p.	Saline		13.7 ± 0.6	14.9 ± 1.0	15.1 ± 1.3	15.2 ± 1.6
	Caffeine	5	15.2 ± 0.8	26.7 ± 1.5*	21.7 ± 1.9*	20.3 ± 2.1**
CGP 35348 100 mg · kg^{-1} i.p.	Saline		13.9 ± 0.9	11.6 ± 1.1**	12.7 ± 1.7	13.1 ± 1.5
	Caffeine	5	15.1 ± 1.0	25.3 ± 1.7*	23.3 ± 2.1*	18.5 ± 1.6
α-MpT 100 mg kg^{-1} i.p.	Saline		14.5 ± 0.7	16.7 ± 1.3	15.8 ± 2.1	15.1 ± 1.9
	Caffeine	5	13.6 ± 0.9	26.4 ± 2.0*	24.5 ± 1.8*	16.8 ± 1.3

The number of mice is shown in parentheses. * $P < 0.01$; ** $P < 0.05$ in comparison with saline-saline; *** $P < 0.01$ versus saline-caffeine treated mice. The number of mice ranged from 8 to 21 with the exception of saline-saline which numbered 27

Table 2 Antinociception exerted by caffeine in the paw-pressure test in the rat and its antagonism by HC-3

Pretreatment (i.c.v.)	Treatment (i.p.)	Dose mg kg^{-1}	Before pretreatment	Paw-pressure (g) After treatment		
				15 min	30 min	45 min
Saline 10 µl/rat	Saline		60.1 ± 3.2 (8)	59.6 ± 3.0 (8)	58.4 ± 3.6 (8)	61.6 ± 2.2 (8)
Saline 10 µl/rat	Caffeine	1	56.9 ± 3.2 (6)	82.6 ± 4.4** (6)	70.6 ± 4.0** (6)	63.4 ± 3.2 (6)
	Caffeine	5	58.4 ± 2.7 (8)	142.3 ± 5.4* (8)	111.3 ± 5.4* (8)	70.6 ± 3.2 (8)
HC-3 1 µg/rat	Saline		56.4 ± 2.8 (10)	61.2 ± 4.1 (10)	62.3 ± 3.7 (10)	59.8 ± 3.4 (10)
	Caffeine	5	61.3 ± 3.1 (7)	69.2 ± 4.8*** (7)	63.5 ± 3.7*** (7)	56.7 ± 4.2 (7)

The number of rats is shown in parentheses.
* $P < 0.01$, ** $P < 0.05$ in comparison with saline-saline; *** $P < 0.01$ versus saline-caffeine treated rat

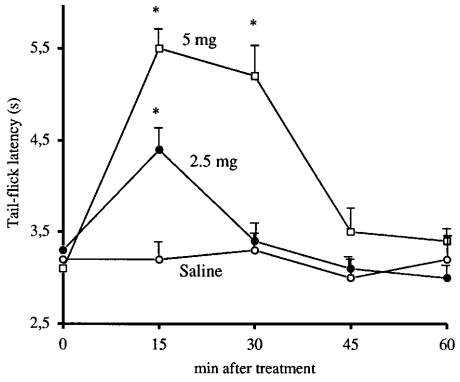

Fig. 4 Antinociceptive effect of caffeine in the rat tail-flick test. *Vertical lines* show SE mean. * $P < 0.01$ in comparison with saline controls. Each point represents the mean of at least 6 rats

2). In the paw-pressure test, no antinociception by caffeine (5 mg kg^{-1} i.p.) was detected in rats pretreated with HC-3 (Table 2). The analgesic profile of caffeine (2.5–5 mg kg^{-1}) investigated in the tail flick test exhibited a similar antinociceptive profile to that observed in the paw pressure test (Fig. 4).

Finally, it should be noted that caffeine elicited its antinociceptive effects without changing motor coordination. Furthermore, caffeine, unlike oxotremorine and physostigmine, increased the pain threshold without causing the typical cholinergic symptomatology (Table 3).

Discussion

Caffeine antinociception was elicited with all noxious stimuli used: thermal (hot-plate and tail flick test), chemical (abdominal constriction test) and mechanical (paw

Table 3 Effect of caffeine in comparison with oxotremorine and physostigmine in: a) the Irwing test; b) rota-rod test

a Irving test

	Tremors	Salivation	Lacrimation	Diarrhoea	Abdominal tone
Saline s.c.	0	0	0	0	4
Caffeine 5 mg kg^{-1} s.c.	0	0	0	0	4
Oxotremorine 100 µg kg^{-1} s.c.	4	4	+	+	0
Physostigmine 200 µg kg^{-1} s.c.	2	6	+	+	2

Tremors:	absent = 0		maximum score = 8
Salivation:	absent = 0		maximum score = 8
Lacrimation:	absent = 0		present +
Diarrhoea:	absent = 0		present +
Abdominal Tone:	flaccid abdomen = 0	normal = 4	abdomen board-like = 8

Each value represents the mean of 5 mice

b Endurance time on rota-rod (s)

	Before treatment	After treatment		
		15 min	30 min	45 min
Saline s.c.	98.6 ± 5.2 (15)	94.5 ± 5.2 (15)	104.6 ± 4.4 (15)	94.8 ± 5.4 (15)
Caffeine 5 mg kg^{-1} s.c.	98.7 ± 5.4 (10)	107.6 ± 5.3 (10)	104.2 ± 5.1 (10)	95.3 ± 5.2 (10)
Oxotremorine 40 µg kg^{-1} s.c.	101.7 ± 7.3 (15)	73.7 ± 6.6* (15)	60.2 ± 7.3* (15)	63.6 ± 7.5* (15)
Physostigmine 200 µg kg^{-1} s.c.	95.6 ± 5.5 (13)	64.8 ± 6.1*	55.5 ± 7.2* (13)	50.6 ± 8.0* (13)

* $P < 0.05$ in comparison with saline controls. The number of mice is shown in parentheses

pressure test). Doses which increase the pain threshold were devoid of any other modification of animal behaviour, such as motor incoordination, as demonstrated by the Irwing and rotarod tests.

Caffeine antinociception was found to be dependent on cholinergic activation as this analgesia is antagonised by the muscarinic antagonists atropine and pirenzepine and by the ACh depletor HC-3. Caffeine exerts its antinociceptive effect by acting centrally since, after i.c.v. administration, it is able to increase the pain treshold with the same intensity as that obtainable after s.c. administration. The antagonism exerted by i.c.v. injected HC-3 in mice and rats on caffeine induced antinociception, shows that the site of action of caffeine is centrally located. A presynaptic mechanism facilitating cholinergic transmission is involved in caffeine antinociception as revealed by HC-3 antagonism. A postsynaptic mechanism of action can be ruled out since, as reported by Bartolini et al. (1987; 1992), HC-3 was not able to antagonise antinociception induced by agonists of postsynaptic muscarinic receptors such as oxotremorine, McN-A-343 and AF-102B. The integrity of the central cholinergic system is, therefore, fundamental for caffeine antinociception.

A large difference exists between the analgesia induced in animals by caffeine and that induced by direct muscarinic agonists and cholinesterase inhibitors. While caffeine, like all drugs interacting with presynaptic muscarinic auto- (Ghelardini et al. 1990) and heteroreceptors (Ghelardini et al. 1992), produces antinociception without any visible side effects, the direct muscarinic agonists and cholinesterase inhibitors provoke, at the same time, antinociception and a clear cholinergic symptomatology (tremors, sialorrhoea, diarrhoea, lacrimation etc.).

The hypothesis of a presynaptic cholinergic mechanism for caffeine is in agreement with previous results demonstrating, by microdialysis studies, an increase in ACh release from rat hippocampus obtained through a selective interaction of caffeine with A_1 receptors (Carter et al. 1995). Since the A_1 agonist N^6-cyclopentyladenosine exerted a counteractive effect not only on caffeine-induced ACh release (Carter et al. 1995), but also on caffeine antinociception, the hypothesis of a presynaptic cholinergic mechanism mediated via A_1 receptor antagonism for caffeine enhancement of the pain threshold is confirmed.

It has been shown that caffeine increases turnover of ACh (Haubrich et al. 1981; Murray et al. 1982) via a mechanism exerted at the level of cholinergic cell bodies (Rainnie et al. 1994). Therefore, the activation of the cholinergic system responsible for the caffeine antinociception could be caused not only by an enhancement of the ACh release, but also by an increased ACh turnover.

The results obtained have shown a discrepancy between the antinociceptive effect of caffeine and the potentiation of cerebral ACh release described by Carter et al. (1995). In fact, the latency required to reach the maximum ACh release (80 min) by caffeine was greater than that required to reach their antinociceptive peak (15–30 min). The greater latency required could be ascribed to the different administration routes (per os in microdialysis studies; i.p. and s.c. for antinociceptive studies). Moreover, the time taken by ACh to diffuse from the synaptic cleft to the microdialysis tube can further increase the latency required to reach the caffeine's maximum effect. The ACh release induced by caffeine was longer-lasting than its analgesic effect. It should be kept in mind that microdialysis experiments were in the presence of neostigmine which, by inhibiting the degradation of ACh, prolongs the increase of extracellular ACh due to caffeine.

Other neurotransmitter systems did not appear to be involved in caffeine antinociception since the opioid antagonist naloxone, in agreement with Sawynok et al. (1995), the $GABA_B$ antagonist CGP 35348, α-methyl-p-tyrosine methyl ester and reserpine, were all unable to prevent a caffeine-induced analgesic effect. In contrast, Sawynok and Reid (1996b) showed that the central depletion of serotonin inhibited caffeine antinociception in the rat formalin test. In our experimental conditions, doses of caffeine about 10-fold lower than those used by Sawynok and Reid and devoid of any other behavioural effect, were employed. Therefore, we cannot exclude the involvement of the serotoninergic system in caffeine antinociception at doses at which the locomotor activity is stimulated.

The doses and administration schedules of naloxone, CGP 35348, α-methyl-p-tyrosine and reserpine were suitable for preventing antinociception induced respectively by morphine (Ghelardini et al. 1990), $GABA_B$ agonist baclofen (Malcangio et al. 1991), amphetamine (Bartolini et al. 1987) and the antidepressant drugs clomipramine and amitriptyline (Galeotti et al. 1995).

In summary, our results show that caffeine antinociception is induced by potentiating endogenous cholinergic activity.

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References

Bartolini A, Galli A, Ghelardini C, Giotti A, Malcangio M, Malmberg-Aiello P, Zucchi PL (1987) Antinociception induced by systemic administration of local anaesthetics depends on a central cholinergic mechanism. Br J Pharmacol 92: 711–721

Bartolini A, Ghelardini C, Gualtieri F, Malcangio M, Malmberg-Aiello P, Romanelli N, Giotti A (1989) AFDX-116 induces analgesia only when injected at very low doses. Trends Pharmacol Sci [Suppl IV]: 99

Bartolini A, Ghelardini C, Fantetti L, Malcangio M, Malmberg-Aiello P, Giotti A (1992) Role of muscarinic receptor subtypes in central antinociception. Br J Pharmacol 105: 77–82

Carter AJ, O'Connor WT, Carter MJ, Ungerstedt U (1995) Caffeine enhances acetylcholine release in the hippocampus in vivo by a selective interaction with adenosine A_1 receptors. J Pharmacol Exp Ther 273:637–642

Cass IJ, Frederik WS (1962) The augmentation of analgesic effect of aspirin with phenacetin and caffeine. Curr Ther Res 4: 583–588

Curatolo PW, Robertson D (1983) The health consequences of caffeine. Ann Int Med 98: 641–653

D'amour FE, Smith DL (1941) A method for determining loss of pain sensation. J Pharmacol Exp Ther 72:74–79

Forbes JA, Beaver WT, Jones KF, Kehm CJ, Smith WK, Gongloff CM, Zeleznock JR, Smith JW (1991) Effect of caffeine on ibuprofen analgesia in postoperative oral surgery pain. Clin Pharmacol Ther 49: 674–684

Galeotti N, Ghelardini C, Bartolini A (1995) Involvement of the serotoninergic system in the analgesic effect of tryciclic antidepressants. Behav Pharmacol 6 [Suppl. 1]: 20

George R, Haslett WL, Jendel DJ (1962) The central action of metabolite of tremorine. Life Sci 1: 361–363

Ghelardini C, Malmberg-Aiello P, Giotti A, Malcangio M, Bartolini A (1990) Investigation into atropine-induced antinociception. Br J Pharmacol 101: 49–54

Ghelardini C, Giotti A, Gualtieri F, Matucci R, Romanelli MN, Scapecchi S, Teodori E, Bartolini A (1992) Presynaptic auto- and hetero-receptors in the cholinergic regulation of pain. In: Angeli P, Gulini U, Quaglia W (eds) Trends in receptor research. Elsevier, Amsterdam, pp 95–114

Gualtieri F, Ghelardini C, Giotti A, Malcangio M, Malmberg-Aiello P, Bartolini A (1989) Analgesia induced by the M_2 antagonist methoctramine administered i.c.v. Trends Pharmacol Sci [Suppl IV]: 99

Haley TJ, McCormick WG (1957) Pharmacological effects produced by intracerebral injections of drugs in the conscious mouse. Br J Pharmacol Chemother 12: 12–15

Harris LS, Dewey WL, Howes J, Kennedy JS, Pars H (1969) Narcotic antagonists analgesics: interaction with cholinergic system. J Pharmacol Exp Ther 169: 17–22

Haubrich DR, Williams M, Yarbrough GG, Wood PL (1981) 2-chloroadenosine inhibits brain acetylcholine turnover in vivo. Can J Pharmacol 59: 1196–1198

Hendershot LC, Forsait J (1959) Antagonism of the frequency of phenylquinone-induced writhing in the mouse by weak analgesics and nonanalgesics. J Pharmacol Exp Ther 125: 237–240

Herz A (1962) Wirkungen des Arecolins auf das Zentralnervensystem. Naunyn-Schmiedebergs Arch Exp Pathol Pharmakol 242: 414–429

Ireson JD (1970) A comparison of the antinociceptive action of cholinomimetic and morphine-like drugs. Br J Pharmacol 40: 92–101

Irwing S (1966) Comprensive observational assessment: Ia. A systematic, quantitative procedure for assessing the behavioral and physiologic state of mouse. Psychopharmacology 13: 222–257

Jackisch R, Strittmatter H, Kasakov I, Hertting G (1984) Endogenous adenosine as a modulator of hippocampal acetylcholine release. Naunyn-Schmiedeberg's Arch Pharmacol 327: 319–325

Jain AK, McMahon FG, Ryan JR, Unger D, Richard W (1978) Aspirin and aspirin-caffeine in postpartum pain relief. Clin Pharmacol Ther 24: 69–75

Jhamandas K, Sawynok J, Sutak M (1978) Antagonism of morphine action on brain acetylcholine release by methylxantines and calcium. Eur J Pharmacol 49:309–312

Koster R, Andersen M, De Beer EJ (1959) Acetic acid for analgesic screening. Fed Proc 18: 412

Laska EM, Sunshine A, Mueller F, Elvers WB, Siegel C, Rubin A (1984) Caffeine as an analgesic adjuvant. J Am Med Assoc 251: 1711–1718

Leighton GE, Rodrigues RE, Hill RG, Hughes J (1988) k-opioid agonist produce antinociception after i.v. and i.c.v. but not intrathecal administration in the rat. Br J Pharmacol 93: 553–560

Lenke D (1958) Narkosepotenzierende und Analgetische Wirkung von 1,4-dipyrrolidino-2-butin Naunyn-Schmiedeberg's Arch Exp Pathol Pharmakol 234: 35–45

Lentz TL, Liley L, Michaelson U (1969) Some action of anticholinergic drugs. Br J Pharmacol 32:156–162

Malcangio M, Ghelardini C, Giotti A, Malmberg-Aiello P, Bartolini A (1991) CGP-35348, a new GABA-B antagonist prevents antinociception and muscle-relaxant effect induced by baclofen. Br J Pharmacol 103: 1303–1308

Malec D, Michalska E (1988) The effect of methylxanthines on morphine analgesia in mice and rats. Pol J Pharmacol Pharm 40: 223–232

Max B (1986) This and that: Please pass the coffee. Trends Pharmacol Sci 7: 12–14

Mirsa AL, Pontani RB, Vadlamani NL (1985) Potentiation of morphine analgesia by caffeine. Br J Pharmacol 84:789–791

Murray TF, Blaker WD, Cheney DL, Costa E (1982) Inhibition of acetylcholine turnover rate in rat hippocampus and cortex by intraventricular injection of adenosine analogs. J Pharmacol Exp Ther 222: 550–554

O'Callaghan JP, Holtzman SG (1976) Prenatal administration of morphine to the rat: tolerance to the analgesic effect of morphine in the offspring. J Pharmacol Exp Ther 197: 533–544

Pedata F, Pepeu G, Spignoli G (1984) Biphasic effect of methylxanthines on acetylcholine release from electrically-stimulated brain slices. Br J Pharmacol 83: 69–73

Pedata F, Giovannelli L, De Sarno P, Pepeu G (1986) Effect of adenosine, adenosine derivatives and caffeine on acetylcholine release from brain synaptosomes: interaction with muscarinic autoregulatory mechanisms. J Neurochem 46 (5): 1593–1598

Pedigo NW, Dewey WL, Harris LS (1975) Determination and characterization of the antinociceptive activity of intracerebroventricularly administered acetylcholine. J Pharmacol Exp Ther 193: 845–852

Person DL, Kissin I, Brown PT, Xavier AV, Vinik HR, Bradley EL (1985) Morphine-caffeine analgesic interaction in rats. Anesth Analg 64: 851–856

Phillis JW, Jiang ZG, Chelack BH, Wu PH (1980) The effect of morphine on purine and acetylcholine release from rat cerebral cortex: evidence for a purinergic component in morphine's action. Pharmacol Biochem Behav 13:421–427

Rainnie DG, Grunze HCR, McCarley RW, Greene RW (1994) Adenosine inhibition of mesopontine cholinergic neurones: implications for EEG arousal. Science 263: 689–692

Sawynok J, Yaksh T (1993) Caffeine as an analgesic adjuvant: a review of pharmacology and mechanism of action. Pharmacol Rev 45: 43–85

Sawynok J, Reid A (1995) Caffeine antinociception: role of formaline concentration and adenosine A_1 and A_2 receptors. Eur J Pharmacol 298:105–111

Sawynok J, Reid AR, Doak GJ (1995) Caffeine antinociception in the rat hot-plate and formaline tests and locomotor stimulation: involvement of noradrenergic mechanisms. Pain 61: 203–207

Sawynok J, Reid A (1996a) Caffeine antinociception: role of formalin concentration and adenosine A_1 and A_2 receptors. Eur J Pharmacol 298: 105–111

Sawynok J, Reid A (1996b) Neurotoxin-induced lesions to central serotoninergic, noradrenergic and dopaminergic systems modify caffeine-induced antinociception in the formalin test and locomotor stimulation in rats. J Pharmacol Exp Ther 277:646–653

Schachtel BP, Fillingim JM, Lane AC, Thoden WR, Baybutt RI (1991) A double-blind study comparing aspirin with caffeine to aspirin and placebo in patients with sore throat. Arch Int Med 151: 733–737

Seegers AJM, Jager LP, Zandberg P, Van Noordwijk J (1981) The anti-inflammatory, analgesic and antipyretic activities of non-narcotic analgesic drug mixtures in rats. Arch Int Pharmacodyn 251: 237–254

Siegers CP (1973) Effect of caffeine on the absorption and analgesic efficacy of paracetamol in rats. Pharmacology 10: 19–27

Spignoli G, Pedata F, Pepeu G (1984) A_1 and A_2 adenosine receptors modulates acetylcholine release from brain slices. Eur J Pharmacol 97: 341–342

Vinegar R, Truax JF, Selph JL, Welch RM, White HL (1976) Potentiation of the anti-inflammatoty and analgesic activity of aspirin by caffeine in the rat. Soc Exp Biol Med 151: 556–560

Ward N, Whitney C, Avery D, Dunner D (1991) The analgesic effects of caffeine in headache. Pain 44:151–155