

Analgesic effect of caffeine and clomipramine: A possible interaction between adenosine and serotonin systems

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The goals of this study were to determine whether the nonselective adenosine receptor antagonist caffeine exerts an analgesic effect and to investigate the time-dependent influence of the selective serotonin reuptake inhibitor clomipramine on the action of caffeine. Results suggest a possible interaction between serotonin and adenosine systems, which may contribute to the analgesic action of drugs. Therefore, the hot-plate and formalin tests were employed in order to measure the response to painful thermic and chemical stimuli. Results have shown that caffeine (1.67, 16.7 and 67 mg kg⁻¹, *i.p.*) exerts a direct dose-dependent analgesic action. When caffeine (1.67 and 16.7 mg kg⁻¹) was combined with clomipramine (3 mg kg⁻¹ *i.p.*), an enhanced analgesic effect was obtained. However, the same combinations were ineffective in a subacute model. In this model, clomipramine was administered for 14 days and the respective dose of caffeine was added on the last day. Therefore, it can be concluded that the serotonin system interacts with the analgesic action of caffeine and that a long-term use of clomipramine probably triggers subsensitivity of adenosine receptors.

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Caffeine, 1,3,7-trimethylxanthine, is a nonselective A₁- and A₂-adenosine receptor antagonist. In the past 20 years an increasing number of controlled clinical studies have produced evidence that it contributes to amelioration of certain pain states (1). Caffeine has been demonstrated to have adjuvant analgesic properties when combined with non-steroidal anti-inflammatory drugs in a number of human pain paradigms (2). Caffeine alone produces an intrinsic antinociceptive action in pain thresholds and in inflammatory tests. Elevation of nociceptive thresholds and enhancement of antinociception may reflect multiple components of the caffeine action. These may be: 1. antagonism of

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the peripheral pronociceptive and hyperalgesic effects of adenosine, 2. activation of central noradrenergic pathways which regulate the nociceptive threshold, and 3. a central psychomotor stimulant action of caffeine (1).

It seems likely that, besides adenosine and the noradrenergic system, a different neurotransmitter systems may be involved in the analgesic action of caffeine. It would be interesting to investigate whether caffeine augments analgesic effect when combined with other potential analgesics.

It is well known that some antidepressants, particularly clomipramine, are used to relieve chronic pain syndromes (3). Although numerous experimental studies have been performed to clarify the mechanism by which antidepressants exert an analgesic action, it is still unclear. The standard, but unsatisfactory, explanation is that they act on descending tracts from the brain *via* noradrenalin and serotonin systems to modulate pain signaling in the spinal cord (4). Clomipramine has a strong and relatively specific capacity to inhibit the reuptake of serotonin and it is thought to be important for its analgesic action (5, 6).

Many preclinical and clinical studies have demonstrated that repeated administration of tricyclic antidepressants (TCAs) is associated with an adaptive change in the sensitivity of receptors for different neurotransmitters in the central nervous system. Namely, β - and α_2 -adrenoreceptors as well as 5-HT₂-receptors are down-regulated (6), while some dopaminergic receptors are up-regulated (7). However, it was recently indicated that an adenosinergic system could also be involved in the effects of some antidepressants (8–10).

Therefore, the goals of the present work were to examine the analgesic action of caffeine and to determine the influence of acute and subacute clomipramine treatment on this effect.

EXPERIMENTAL

Animals

Male black mice (C₅₇) weighing about 30 g were used in the experiments. Animals were housed in groups in colony cages with free access to food and water, except during experiments. They were maintained in climate- and light-controlled rooms (21 ± 1 °C, relative humidity about 50%, 12 h dark/light cycle). Experiments took place between 9:00 am and 3:00 pm. All animal procedures were carried out pursuant to the Croatian Law of Animal Welfare and were approved by The Ministry of Science and Technology of the Republic of Croatia.

Drugs

Caffeine (caffeine-sodium benzoate, Sigma, USA) and clomipramine (Anafranil, Pliva, Croatia) were dissolved in 0.9% NaCl. Solutions of caffeine (0.1, 1, and 4 g L⁻¹) and clomipramine (180 mg L⁻¹) were injected intraperitoneally (*i.p.*) in a volume of 0.5 mL *per* 30 g body mass. Control animals were given an equal volume of saline. Injections were given 30 minutes before nociceptive testing.

For the acute combination treatment, clomipramine was injected 30 min before caffeine and antinociceptive tests took place 30 min after the last injection.

In the subacute model with clomipramine, the latter was administered *i.p.* once daily for 14 subsequent days and antinociceptive measurements were taken 30 minutes after the 14th injection. For the combination treatment, the respective dose of caffeine was applied 30 minutes after the last clomipramine injection, and testing was performed after 30 min.

Nociceptive testing

Formalin and hot-plate tests were employed to measure the reactions to noxious stimuli 30 minutes after saline administration or drug treatment (caffeine, clomipramine or combination treatment). Each experimental group consisted of eight animals.

Constant temperature hot-plate test (11). – Surface temperature of the plate was maintained at 52 ± 1 °C. Mice from each group were individually placed on the plate and the latency of the response to a thermal noxious stimulus, such as paw lick or jumping, was recorded. A cut-off time of 45 s was employed.

The thermic stimulus employed in the constant temperature hot-plate test is strong and elicits a response within a few seconds (11, 12). This short-lasting stimulus does not cause any damage to paw tissue, so it can be followed by the formalin test.

Formalin test (13). – 1% solution of formalin in 0.9% NaCl was injected subcutaneously (20 μ L) into the dorsal surface of the right fore-paw of the mice using a microsyringe 26-gauge needle. Animals were then placed in a plexiglas chamber and observed for nociceptive behavior during 180 s. The time mice spent licking the injected paw was recorded as the reaction to the chemical stimulus.

The stimulus employed in the formalin test is chemical and elicits a long lasting response. The formalin test provides a model of the behavioral response to a moderate tonic, inflammatory pain and the nociceptive stimulus used in this test most clearly resembles some of the stimuli found in patients with clinical pain (12, 13).

These two tests were carried out in the same session, the hot-plate test being followed by the formalin test.

Statistical analysis

Results are presented as mean values \pm SEM. Mean drug-induced differences were analyzed using the analysis of variance (ANOVA), followed by the Newman-Keuls test for multiple groups.

RESULTS AND DISCUSSION

Analgesic actions of caffeine and clomipramine

Results obtained by the hot-plate test have shown that caffeine exerts an analgesic effect in a dose-dependent manner. Although the latency time for caffeine at doses of 1.67 and 16.7 mg kg⁻¹ b.m. was longer compared to saline-treated animals, significant results were obtained only with the highest dose of 67 mg kg⁻¹ ($p < 0.001$) (Table I). In the

formalin test, caffeine at 16.7 and 67 mg kg⁻¹ showed significantly shorter licking times compared to the controls ($p < 0.05$ and $p < 0.001$, respectively), while the lowest dose (1.67 mg kg⁻¹ b.m.) was ineffective (Table I). For comparison, a dose of 1.67 mg kg⁻¹ b.m. corresponds to an average caffeine concentration in one cup of coffee (40–180 mg per 150 mL coffee).

Caffeine has previously been demonstrated to produce antinociception in different threshold tests at higher doses (> 100 mg kg⁻¹ b.m.) than those used in the present testing (2, 11). Sawynok *et al.* (14) determined caffeine antinociception in the rat hot-plate and formalin tests. In this study, a predominant central rather than peripheral site of action of caffeine was suggested because its peripheral coadministration with formalin did not produce antinociception.

Table I. Analgesic effects of caffeine^a

Drug treatment	Dose (mg kg ⁻¹)	Latency time (s)	Licking time (s)
Control (saline)	–	4.2 ± 0.4	75.1 ± 2.7
Caffeine	1.67	4.6 ± 0.6	80.4 ± 3.7
	16.7	5.0 ± 0.6	61.3 ± 2.9 ^b
	67.0	9.5 ± 0.9 ^c	46.9 ± 1.9 ^c

^a Mean ± SEM, $n = 8$.

Statistically significant difference *versus* control group: ^b $p < 0.05$, ^c $p < 0.001$.

Caffeine has repeatedly been shown to enhance the turnover of noradrenaline in a number of brain regions. This action could result from a blockade of tonic inhibition of neurons in the locus coeruleus by adenosine as well as blockade of inhibitory adenosine receptors on central noradrenergic nerve terminals by caffeine. Thus, the ability of caffeine to increase noradrenaline turnover in multiple brain regions and the spinal cord could contribute to antinociception and to adjuvant properties of caffeine (11).

The antidepressant clomipramine (3 mg kg⁻¹ b.m.) was administered once (acute test) and repeatedly during 14 days (subacute test). No analgesic effect of a single clomipramine dose was observed either in the hot-plate or in the formalin test (Table II). Similar results were obtained in the subacute model.

Table II. Analgesic testing of acute and subacute clomipramine^a

Drug treatment	Dose (mg kg ⁻¹)	Latency time (s)	Licking time (s)
Control (saline)	–	4.2 ± 0.4	75.1 ± 2.7
Clomipramine (1 ×)	3.0	4.8 ± 0.3	71.6 ± 3.3
Clomipramine (14 ×)	3.0	4.6 ± 0.3	67.9 ± 4.3

^a Mean ± SEM, $n = 8$.

Although the results of this testing did not show any antinociceptive effect of clomipramine, it is well known that serotonin reuptake inhibitors relieve pain syndromes (3). Fasmer *et al.* (5) determined the antinociceptive effect of these drugs in mice using higher doses, so it seems that the analgesic action of antidepressants is dose-dependent.

Analgesic action of caffeine in combination with acute and subacute clomipramine

Although drugs *per se* were ineffective, their combination showed quite different results, both in acute and in subacute experiments (Table III). Namely, in the hot-plate test the latency time for combination treatments with caffeine (1.67 and 16.7 mg kg⁻¹ b.m.) was significantly longer, while the licking time in the formalin test was shorter compared to drugs alone in acute tests ($p < 0.001$). Therefore, the results showed a significant analgesic effect when clomipramine was combined with caffeine.

Clomipramine was administered before caffeine in order to obtain its full pharmacodynamic effect before caffeine application. The metabolism of clomipramine is slow and a considerable accumulation of pharmacologically active metabolite, desmethylclomipramine, has been observed in lipid membranes (6, 15).

The observed synergistic analgesic effect of caffeine combined with clomipramine, obtained in this pharmacological investigation, might be due to a potential interaction between the central serotonin and adenosine neurotransmitter systems.

A possible involvement of the serotonin system in the analgesic effect of caffeine also supports the results obtained for the combination of caffeine with subacutely (14 days) administered clomipramine. However, after subacute use of antidepressant, the synergistic analgesic effect of caffeine was markedly produced compared to the same combination with acute clomipramine. In the latter case the time values were similar as for drugs alone (Table III).

The observed discrepancies could be due to potential adaptations in the adenosine system, *e.g.*, the subsensitivity of receptors triggered by a long-term use of the antidepressant.

Table III. Analgesic effect of caffeine in combination with acute and subacute clomipramine^a

Drug treatment	Dose (mg kg ⁻¹)	Latency time (s)	Licking time (s)
Control (saline)	–	4.2 ± 0.4	75.1 ± 2.7
Clomipramine (1 ×)	3	6.1 ± 0.4 ^b	48.5 ± 2.4 ^d
+ caffeine	1.67		
Clomipramine (1 ×)	3	6.4 ± 0.3 ^c	47.4 ± 3.6 ^d
+ caffeine	1.67		
Clomipramine (14 ×)	3	3.5 ± 0.3	75.6 ± 5.2
+ caffeine	1.67		
Clomipramine (14 ×)	3	4.2 ± 0.3	69.3 ± 5.8
+ caffeine	1.67		

^a Mean ± SEM, $n = 8$.

Statistically significant difference *versus* control group: ^b $p < 0.05$, ^c $p < 0.01$, ^d $p < 0.001$.

CONCLUSIONS

In summary, the results of this behavioral analysis provide evidence that: 1. caffeine exerts an analgesic effect that is dose-related; 2. when caffeine is combined with the antidepressant clomipramine, a synergistic analgesic effect is obtained; 3. the same combination with repeated application of clomipramine fails to produce an analgesic effect probably because of the observed subsensitivity of adenosine receptors.

It can be assumed that an interaction between the central serotonin and adenosine systems exists and that it is involved in the analgesic action of drugs such as caffeine and clomipramine.

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S A Ž E T A K

Analgetsko djelovanje kofeina i klomipramina: moguća interakcija između adenozienskog i serotoniniskog sustava

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U ovom radu ispitivano je analgetsko djelovanje neselektivnog antagonista adenozienskih receptora, kofeina, u dozama od 1,67, 16,7 i 67 mg kg⁻¹ tjelesne mase te je promatran utjecaj jednokratne i višekratne primjene antidepresiva klomipramina (3 mg kg⁻¹ tjelesne mase), selektivnog inhibitora ponovnog povrata serotoina, na djelovanje kofeina. U tu svrhu korištena su dva eksperimentalna modela, metoda vruće ploče i ispitivanje s formalinom, koja termičkim, odnosno kemijskim, stimulansom izazivaju osjet boli kod miševa. Rezultati su pokazali da kofein ima analgetski učinak koji je upravo proporcionalan dozi.

Kod kombinacije kofeina (1,67 i 16,7 mg kg⁻¹ tjelesne mase) s jednokratno primijenjenim klomipraminom, analgetsko je djelovanje bilo značajno jače u odnosu na učinke lijekova pojedinačno. Međutim, iste kombinacije kofeina s višekratno (14 dana) primijenjenim klomipraminom nisu djelovale analgetski. To bi moglo biti posljedicom promijenjene osjetljivosti adenozienskih receptora uslijed višekratne primjene antidepresiva. Stoga bi se na osnovu obavljenih ispitivanja u ovom radu mogla pretpostaviti uključenost središnjeg serotoniniskog sustava u analgetsko djelovanje kofeina.

Ključne riječi: kofein, klomipramin, analgetsko djelovanje, metoda vruće ploče, ispitivanje s formalinom, adenozienski sustav, serotoniniski sustav

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