Pharmacology Biochemistry and Behavior, Vol. 45, pp. 791–796, 1993
Printed in the U.S.A.

0091-3057/93 $6.00 + .00

Effects of a Subacute Treatment in Rats by a Fresh Cola Extract on EEG and Pharmacokinetics

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Received 20 April 1992

VAILLE, A., G. BALANSARD AND G. JADOT. *Effects of a subacute treatment in rats by a fresh cola extract on EEG and pharmacokinetics.* PHARMACOL BIOCHEM BEHAV 45(4) 791–796, 1993.—The compared effects of an acute and subacute treatment by fresh cola extract and caffeine on the caffeine pharmacokinetics and on cortical activities by spectral analysis of the electroencephalogram (EEG) are studied in rats. After acute cola administration, we observed an increase in half-life elimination of caffeine and a stabilization of its plasma/erythrocyte ratio. Chronic administration revealed differences in cola–caffeine penetration in erythrocytes and a reduction of the area under the curve (AUC) and plasma/erythrocyte ratio. We also noted a significant difference in the binding of the caffeine on plasma proteins after subacute administration of cola seed extract. Cola seed treatment induces an increase in the cortical activity with a widening of the dominant frequency spectrum 7- to 10-Hz band of EEG, whereas caffeine alone induces a shift of the dominant frequency band toward higher frequencies. The observed delay to obtain the greatest EEG effect related to the caffeine contained in cola seeds can be partially explained by the pharmacokinetic data.

Caffeine Cola seeds Psychostimulant EEG Pharmacokinetics Rats

NOMENCLATURE

AUC_{exp}	area under the experimental curve
T_{max1}	time of the maximum concentration of the first phase
T_{max2}	time of the maximum concentration of the second phase
C_{max1}	maximum concentration of the first phase
C_{max2}	maximum concentration of the second phase
K_{abs}	apparent constant of absorption
K_{el}	apparent constant of elimination
$T_{1/2abs1}$	half-life time of the first absorption phase
$T_{1/2abs2}$	half-life time of the second absorption phase
$T_{1/2el1}$	half-life time of the first elimination phase
$T_{1/2el2}$	half-life time of the second elimination phase
V_d	volume of distribution

THE cola seed, *Cola nitida* (Vent.) A. Chev., has been known for a long time in tropical Africa as a stimulant (1) but has only been used in Europe since the late 19th century (4). During the first half of the 20th century, many authors studied the pharmacological action of these seeds (2). The first results, based upon the African tradition, revealed differences in activity between fresh and dry seeds. Such differences were further attributed to the catechin derivatives present in fresh cola seeds. These derivatives in relation with the combination of caffeine and catechine alter its effects (19).

In a previous study, we observed that the behavioral effects on activity and reactivity tests resulting from subacute treatment of fresh cola seeds are more gradual than those of caffeine (27). In this article, we carried out a subacute study of the electroencephalogram (EEG) activity compared with pharmacokinetic parameters.

METHOD

Vegetal Material and Caffeine

The atomized extract of stabilized fresh cola seeds was obtained using Note's method (22). The stabilization was made by boiling ethanol at 88°C under reflux for about 1 h. The dry extract contains the active substances of the fresh seed with the following components: caffeine (6.2%); theobromine (0.9%); catechin (15%); total catechin derivatives (47%). Caf-

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feine (1-3-7-Triméthylxanthine) Carlo Erba was the reference molecule. Fresh extracts and caffeine aqueous solutions were prepared at the beginning of the experiment every day.

We have chosen 20 mg · kg^{-1} as the reference dose of pure caffeine, based upon various behavioral test results, and 320 mg · kg^{-1} cola extract as the equivalent of 20 mg · kg^{-1} pure caffeine.

Animals

The experiments were carried out on 198 adult, male Wistar-AF gnotoxenic rats (average weight 200 ± 20 g) bred by IFFA-CREDO (France). They were housed individually or 10 in a cage, submitted to a natural dark-light cycle, and maintained throughout on food (U.A.R.) and water ad lib.

Pharmacokinetic Study

Drug administration. Animals were randomly divided into four groups:

Two groups of 55 rats receiving acute administration: Group I was treated by 20 mg · kg^{-1} pure caffeine (pH: 4.8 at 37°C) and group II by 320 mg · kg^{-1} cola extract (pH: 5.9 at 37°C).

Two groups of 44 rats receiving subacute administration: Group III was treated by 20 mg · kg^{-1} pure caffeine (pH: 4.8 at 37°C) and group IV by 320 mg · kg^{-1} cola extract (pH: 5.9 at 37°C).

Substances were administered daily per os (1 ml/rat at 37°C) between 9:00 and 12:00 a.m. for 30 days.

Blood sample collection. Blood samples were taken by intracardiac punction using heparin-washed tubes (a) 0.5, 1, 1.5, 2, 3, 6, 7, 8, 10, 12, and 24 h after acute administration and (b) 0.5, 1, 1.5, 2.5, 4, 6, 8, 10, 12, 18, and 24 h after the last day of subacute administration.

After immediate centrifugation (15 min at 60,000 rotations/min), the plasmatic and erythrocytal fractions were separated. The erythrocytes were washed three times in 5 ml NaCl 9‰; and then homogenized and centrifuged. After elimination of the washing solution, the globular phase was frozen for 1 h at −20°C to allow the release of the globular content by rupture of the erythrocytal membranes. At the moment of dosage, the globular samples were put at room temperature and the test sample diluted in 2 ml NaCl 9‰.

In chronically treated animals, brains were, at the same time, removed, rinsed in NaCl 9‰, drained, and then frozen.

Caffeine assays. Caffeine was assayed in blood, plasma, and brain.

Blood. Once the caffeine extracted from the blood samples, its plasma and globular concentrations were determined by high-performance liquid chromatography (29). This technique required a microporasil column (30 cm in length, 3.9 mm in diameter, 10 μm in hole diameter) as a stationary phase and a mixture of hexane–ethanol (85/15) as a mobile phase. The detection was performed using a spectrometer at 280 nm. The chromatograph was a Waters equipped with a 6000-A pump, a UK6 injector, a M480 detector, and a Hitachi-Merck integrator. With this method, the variation coefficients were 3 and 4% for 1 and 10 mg/l, respectively, and the sensitivity on the order of 0.25 mg/l. The free plasma caffeine concentrations were assayed using the Jadot method (16).

Brain. The brain was unfrozen, left to drain for 30 min, weighed to adjoin it four times its volume of 90° alcohol, and ground. Once centrifuged (10 min at 5,000 rotations/min), the caffeine concentration was determined by the EMIT method (immunoenzymatic method) (31).

Pharmacokinetic parameters and statistics. The parameters measured after acute administration [T_{max1}; T_{max2}; C_{max1}; C_{max2}; $T_{1/2abs1}$; $T_{1/2abs2}$; $T_{1/2el1}$; $T_{1/2el2}$; area under the curve $(AUC)_{exp}$] and those measured after subacute administration [T_{max}; C_{max}; K_{abs}; K_{el}; $T_{1/2abs}$; $T_{1/2el}$; AUC_{exp}; V_d; clearance (Cl)] in the plasma and erythrocytal compartments were determined with the help of the IGPHARM software (14), taking into account the two phases of absorption and elimination according to a monocompartmentalized model (two phases).

In addition, the kinetic profile of the free plasma concentrations and the evolution of the plasmatic–erythrocytal concentration ratios were followed. The same step was taken in regard to the concentrations at the whole-brain level. All experimental values (AUC_{exp}, C_{max}, K_{abs}, K_{el}) were analyzed using the Student's *t*-test after comparing variations with the *F*-test. The $T_{1/2}$ were compared using the slope of the linear regression (first sort) after covariance analysis ($p \leq 0.05$) (24).

Electroencephalographic Studies

Animal preparation. Thirty rats (singly housed) were anesthetized with chloral hydrate (350 mg/kg, IP) and placed in a stereotaxic frame (DK 900, David Kopf Instruments, Topanga, CA). Five recording electrodes (stainless steel screws) implanted into the skull to obtain the four cortical bipolar derivations—a)right transversal; b)left transversal; c)frontal; d)occipital—were secured with dental cement (Ivoclar, St. Jorioz, France).

After surgery, rats were allowed to rest for 10 days and randomly divided into 3 groups of 10: The control group (group I) received daily 1 ml distilled water, group II was treated by 20 mg · kg^{-1} pure caffeine, and group III was treated by 320 mg · kg^{-1} cola extract (i.e., 20 mg · kg^{-1} pure caffeine).

Substances were administered PO (1 ml/rat at 37°C) between 9:00 and 12:00 a.m. for 15 days.

EEG recordings. The EEG recording of the four bipolar derivations was performed using an ALVAR type 4 PR electroencephalograph 1 h after drug administration on the 1st, 5th, 10th, and 15th days of treatment and 1 week after its arrest. Before any drug administration, a preliminary recording was performed. On each recording, the frequency of basal rhythms and the presence or absence of epileptic-like spikes were taken into account.

A frequency analysis was done on the 12th day of treatment (when the cola extract reaches its maximum effect) with a Fourier HP 5452 A analyzer using five rats of each group.

After connection to the recording system, for 15 min the animal was allowed to become accustomed to the situation. The EEG spectra obtained by 10 summed-up sweeps were recorded during 5 s each on a 0- to 20-Hz band with a resolution of 0.2 Hz. The recorded results were treated with variance analysis (24).

RESULTS

Pharmacokinetic Study

Acute administration. After oral administration of caffeine solution and cola extract, analysis of the kinetics of the plasma and erythrocyte caffeine concentrations shows the existence of two peaks of concentration of caffeine. However, in caffeine-treated animals these peaks occur much later. Study of the pharmacokinetic parameters, presented in Table

TABLE 1

PHARMACOKINETIC PROPERTIES OF CAFFEINE AFTER ACUTE TREATMENT BY PURE CAFFEINE OR COLA

Parameters	Plasma		Erythrocytes	
	Caffeine	Cola	Caffeine	Cola
T_{max1} (h)	1.5	1	1.5	1
C_{max1} (μg/g)	17.5 ± 1.9	15.5 ± 1.6	11.9 ± 1.7	10.7 ± 0.8
T_{max2} (h)	8	3	7	3
C_{max2} (μg/g)	7.13 ± 1.1	14.3 ± 1.1*	6.83 ± 1.1	8.74 ± 1.0
$T_{1/2ab1}$ (h)	1.66 ± 0.2	1.17 ± 0.1	2.23 ± 0.3	1.06 ± 0.2†
$T_{1/2el1}$ (h)	1.74 ± 0.1	2.97 ± 0.3†	2.06 ± 0.1	2.22 ± 0.3
$T_{1/2ab2}$ (h)	1.90 ± 0.2	5.56 ± 2.1	0.79 ± 0.1	5.23 ± 1.2*
$T_{1/2el2}$ (h)	0.96 ± 0.1	2.44 ± 0.2‡	1.41 ± 0.1	2.69 ± 0.3*
AUC_{exp} (μg/g × h)	82.7 ± 4.6	88.6 ± 5.0	60.8 ± 2.1	56.6 ± 2.6

Data are expressed as mean ± SD.
Statistical signification: comparison of cola vs. caffeine—*$p \leq 0.05$, †$p \leq 0.01$, ‡$p \leq 0.001$.

1, indicates the following results. After administration of caffeine, comparatively to that of the cola extract, we observe the following.

Plasma. * A greatly delayed T_{max1} and T_{max2} (1.5 and 8 h in comparison to 1 and 3 h).

* An identical C_{max1} and a greatly diminished C_{max2} ($p \leq 0.005$).

* Two identical phases of absorption.

* Two phases of elimination significantly shortened.

* An identical AUC_{exp}.

Erythrocytes. * A greatly delayed T_{max1} and T_{max2} (1.5 and 7 h in comparison to 1 and 3 h).

* An identical C_{max1} and C_{max2}.

* A longer first phase of absorption ($p \leq 0.025$) and a shorter second ($p \leq 0.01$).

* An identical first elimination phase but a second phase significantly shortened ($p \leq 0.01$).

* An identical AUC_{exp}.

Chronic administration. After chronic oral administration of caffeine and cola extract, the variations in plasma and erythrocyte caffeine concentrations show a peak of maximal concentration that appears much earlier in animals treated by cola extract. This kinetics is not found at the cerebral level.

The analysis of the pharmacokinetic parameters shown in Table 2 provided the following results.

Plasma level. * A delayed T_{max} (1.5 in comparison to 1 h).

* An identical C_{max}.

* A lengthened absorption phase ($p \leq 0.01$).

* An identical elimination phase.

* An identical AUC_{exp}.

* An identical volume of distribution.

* An identical clearance.

Erythrocyte level. * A delayed T_{max} (2.5 in comparison to 1 h).

* An increased C_{max} ($p \leq 0.01$).

* A longer absorption phase ($p \leq 0.001$).

* A shorter elimination phase ($p \leq 0.001$).

* An increased AUC_{exp} ($p \leq 0.01$).

* A diminished volume of distribution ($p \leq 0.001$).

* A decreased clearance ($p \leq 0.001$).

Brain level. * An identical T_{max}.

* An increased C_{max} ($p \leq 0.001$).

* An identical absorption phase.

* A longer elimination phase ($p \leq 0.001$).

* An increased AUC_{exp} ($p \leq 0.05$).

* A diminished volume of distribution ($p \leq 0.01$).

* A decreased clearance ($p \leq 0.001$).

TABLE 2

PHARMACOKINETIC PROPERTIES OF CAFFEINE DURING SUBACUTE TREATMENT BY PURE CAFFEINE OR COLA

Parameters	Plasma		Erythrocytes		Brain	
	Caffeine	Cola	Caffeine	Cola	Caffeine	Cola
T_{max1} (h)	1.5	1	2.5	1	1	1
C_{max1} (μg/g)	135 ± 11	125 ± 12	126 ± 12	90 ± 4.4*	13.1 ± 0.5	10.5 ± 0.4†
K_{abs} (h^{-1})	0.76 ± 0.01	0.84 ± 0.02*	0.7 ± 0.01	0.86 ± 0.02†	0.82 ± 0.02	0.86 ± 0.01‡
K_{el} (h^{-1})	0.32 ± 0.004	0.33 ± 0.005‡	0.24 ± 0.004	0.21 ± 0.003*	0.17 ± 0.002	0.19 ± 0.001†
$T_{1/2ab}$ (h)	0.91 ± 0.02	0.82 ± 0.03*	1.03 ± 0.01	0.81 ± 0.04‡	0.85 ± 0.04	0.80 ± 0.03
$T_{1/2el}$ (h)	2.13 ± 0.05	2.08 ± 0.04	2.86 ± 0.04	3.30 ± 0.02†	3.94 ± 0.02	3.61 ± 0.01†
AUC_{exp} (μg/g × h)	847 ± 46	882 ± 64	878 ± 72	585 ± 39*	72 ± 8.3	52.7 ± 4.8‡
V_d (l)	74.2 ± 3.6	69.3 ± 4.6	97.5 ± 4.9	168 ± 6.9†	1,618 ± 74	2,026 ± 91*
Cl (l/h)	24.0 ± 0.9	23.1 ± 1.1	23.6 ± 1.2	35.4 ± 1.3†	285 ± 11	389 ± 14†

Data are expressed as mean ± SD.
Statistical signification: comparison of cola vs. caffeine—*$p \leq 0.05$, †$p \leq 0.01$, ‡$p \leq 0.001$.

Electroencephalographic Results

The EEG recordings of animals treated by cola extract were, when compared to those of the control group, more monoform and regular, with equal or greater amplitude, sometimes presenting a desynchronization tendency. The EEG recordings of caffeine-treated animals were particularly desynchronized and microvolted.

No paroxystic spikes have been observed in any group.

As early as the first day of treatment, the basal EEG rhythm of cola extract- or caffeine-treated animals tends to shift significantly toward the high frequencies when compared with the control group. There is less effect in animals treated by cola extract than in those administered caffeine but the differences between these two groups are not statistically significant before the 15th day of treatment. After the end of treatment, no remaining effect was noted (Table 3).

The frequency analysis of the EEG as expressed in % of the total value of the spectral power indicates (Fig. 1):

- a significant increase ($p \leq 0.01$) in the 7-, 9-, and 10-Hz frequency bands in cola extract-treated animals and in the 9-, 10-, and 11-Hz frequency bands in those administered caffeine when compared with the control group.
- a significant increase ($p \leq 0.001$) in the 9- and 10-Hz frequency bands in animals treated by caffeine in comparison to those treated by cola extract.

DISCUSSION

The results of this pharmacokinetic study, after acute and subacute administration, can be analyzed in three phases: absorption, distribution, and elimination.

Absorption

A characteristic of the acute kinetic of caffeine and cola extract is the presence of two plasma peaks. Several authors (10,18) already observed the one peak, which occurs 1.5 h after administration, but the second peak is rarely observed due to the fact that most studies on the kinetics of caffeine have not been prolonged over 8 h or, if so, greatly over this time but at far more important doses.

The existence of this second peak can be related either to a delayed resorption of the caffeine absorbed orally or to a redistribution of the caffeine already resorbed. This redistribution has already been observed but at higher doses (100 mg/kg) that permitted to implicate the influence of caffeine on intestinal absorption (11). Volume, pH, and composition of the solutions in which the caffeine is dissolved could also influence its absorption (8,6). This might explain the difference found concerning the T_{max1} and T_{max2} when caffeine or cola extract is administered.

Distribution

The caffeine distribution depends upon the binding of plasma and erythrocyte proteins. Our study shows a free plasma fraction of 75.4% for caffeine comparable to that of previous papers (7,9,19). Given that its free fraction is of only 54.1%, the binding of caffeine contained in cola extract to the plasma protein is higher. In addition, the free fraction of caffeine released from cola extract undergoes, in time, less important variations than those of pure caffeine (cola, 47–66%; caffeine, 57–87%).

In the erythrocyte compartment, after acute administration this kinetic profile differs from that described at plasma level and particularly concerning C_{max2} and the two absorption phases. Thirty minutes to 9 h after administration, a stabilization of the plasma/erythrocyte ratio was observed. This ratio is similar for caffeine and for cola (caffeine: 1.38 ± 0.6/cola: 1.45 ± 0.05).

After subacute administration, we observed, in comparison with the plasma level, differences in T_{max} (caffeine), C_{max}, elimination phases, AUC_{exp}, V_d, and Cl. As to the plasma/erythrocyte ratio, we note an increase in the average index concerning the cola extract [cola, 1.46 ± 0.22; caffeine, 1.07 ± 0.25 ($p \leq 0.01$)] and resulting in a decrease of the erythrocytal penetration.

Elimination

After acute administration of cola extract, the modifications of the pharmacokinetic parameters, particularly the increase in the $T_{1/2el}$ and stabilization time of the plasma–erythrocyte ratio, allow us to conclude in favor of a stable and prolonged release of caffeine from cola extract.

On the other hand, after subacute administration we have no pharmacokinetic modifications at the plasma level between pure caffeine and the caffeine released from cola extract. The same observation does not apply at the erythrocyte level because the penetration of the caffeine released from cola extract is diminished, which entails a reduction of the AUC_{exp} and the plasma/erythrocyte ratio.

After subacute administration, the binding of the caffeine released from cola extract to plasma proteins is more important with a weaker free fraction and the cerebral impregnation is weaker. This suggests that a clear difference exists between pure caffeine and caffeine released from cola, whose effects, secondary or toxic, are diminished. This is confirmed by the study done at the cerebral level where a much lower C_{max} ($p \leq 0.001$), a slightly longer absorption phase ($p \leq 0.05$),

TABLE 3

EVOLUTION OF BASAL RHYTHMS (Hz) DURING SUBACUTE TREATMENT BY CAFFEINE OR COLA

Groups	Days				
	1	5	10	15	22
Control	7.20 ± 0.20	8.82 ± 0.13	8.10 ± 0.15	8.36 ± 0.11	8.50 ± 0.23
Caffeine	9.20 ± 0.20*	9.90 ± 0.19*	9.12 ± 0.07*	9.18 ± 0.12*	8.62 ± 0.22
Cola	8.85 ± 0.07†	9.60 ± 0.14†	9.15 ± 0.11†	8.25 ± 0.11‡	8.15 ± 0.12

Basal rhythms (Hz) is expressed as frequency mean ± SD. Ten animals in each group.
Statistical signification: comparison ($p \leq 0.05$) – *caffeine vs. control, †cola vs. control, ‡cola vs. caffeine.

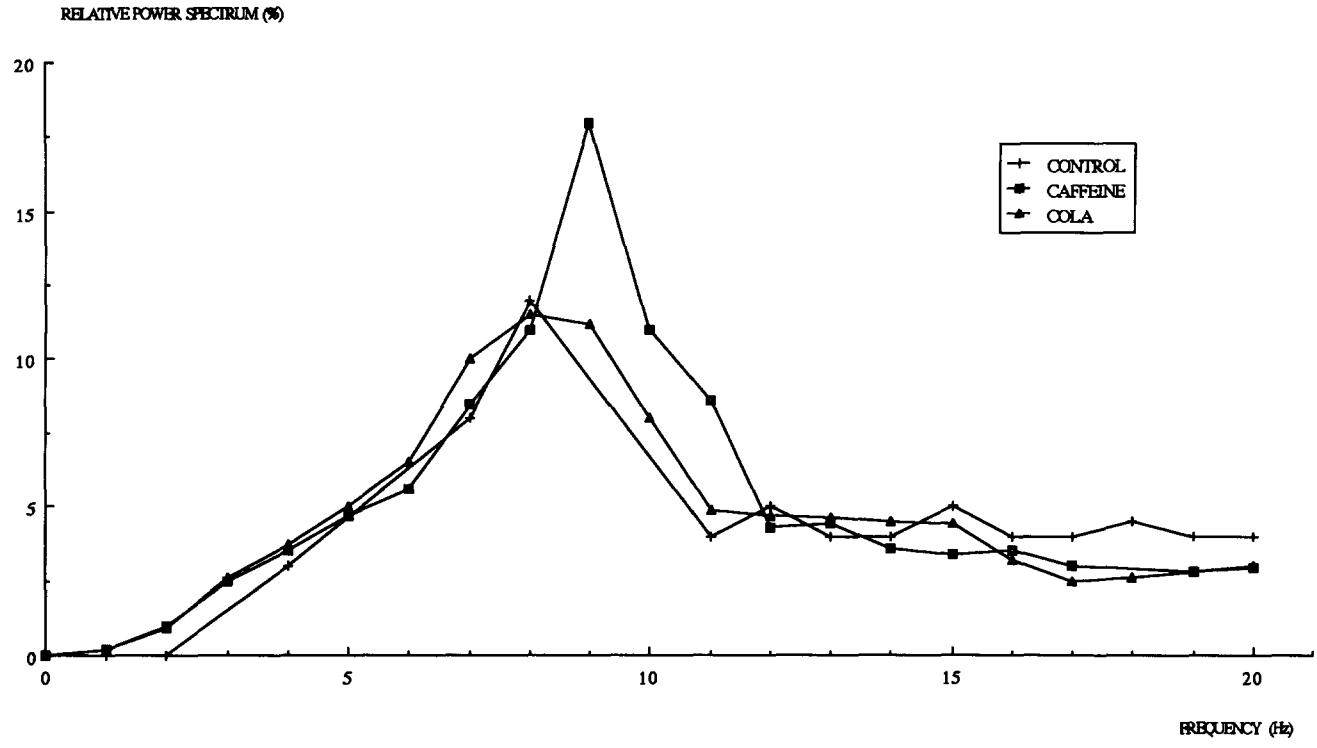

FIG. 1. Percentages of total values of spectral power.

and a greatly shortened elimination ($p \leq 0.001$) were observed.

After the absorption of cola extract, during acute administration the modifications of the pharmacokinetic parameters (particularly the increase in the $T_{1/2el}$ and stabilization time of the plasma/erythrocyte ratio) might induce a stable and prolonged action of the caffeine released from the extract.

After subacute administration, no pharmacokinetic modification was noted at the plasma level between pure caffeine and the caffeine released from the extract. The same observation does not apply at the erythrocytal level because the penetration of the caffeine from the extract is reduced and results in a decrease of the AUC_{exp} and plasma/erythrocyte ratio.

Finally, we note an important decrease in the plasma concentrations not involved in the binding of caffeine to plasma proteins after subacute administration of cola extract. Nevertheless, these concentrations undergo less important variations in time than those observed after subacute administration of caffeine. This may suggest an attenuation of the effects of the caffeine contained in cola extract as well as a reduction in its secondary or toxic effects. This hypothesis is confirmed by the fact that a weaker cerebral impregnation has been found.

EEG Activity

The effects of cola extract administration on EEG activity in rats are mainly characterized by the progressive modification of the cortical activity, inducing a widening of the dominant frequency band and suggesting a more complex spectrum and increase in EEG activity. These effects are different than those obtained with caffeine, which are mainly characterized by a shift of the dominant frequency toward higher frequencies.

The results obtained on EEG activity after caffeine treatment confirm the effect of caffeine. This drug led to an increase in intracerebral noradrenaline, dopamine, and serotonin concentrations (8) and in adenosine concentration (23) by modification of its binding to its receptors (28). This drug causes an activation of the monoaminergic systems altering the characteristics of the EEG (12,26), particularly concerning cortical activity. Our results are in agreement with those obtained after caffeine treatment (5,13,20,25) or by other psychostimulants such as amphetamine (11,17) in various animal species and in man.

Nevertheless, the EEG records do not show significant differences on visual reading between the 1st and 15th days of treatment. This suggests that the habituation phenomenon observed in behavior tests does not occur at the cortical activity level and/or only at the prescribed dose are the effects of the caffeine on EEG maximal from the first day of treatment.

The study of the EEG spectra indicates a significant increase ($p \leq 0.01$) in 7-, 9-, and 10-Hz frequencies in cola extract-treated animals and in 9- and 10-Hz frequencies in those receiving caffeine in comparison to the control group. The different bioavailability of caffeine released from cola extracts can justify the increased response in caffeine-treated animals.

The effects of chronically administered cola extract on EEG activity are undoubtedly partially induced by the action of the caffeine contained in the cola extract.

The delay to obtain a maximum effect on EEG is in agreement with our previous results (27), which showed optimal activity around day 12 in caffeine-treated animals. This delay can be explained as a change in the pharmacokinetic characteristics of the caffeine contained in cola extract under the influence of the catechin.

Thus, after subacute administration of cola extract, the binding of caffeine combined with catechin to plasma proteins is more marked than that with caffeine administration alone (19). This induces a lower caffeine intracerebral concentration during the first days of treatment.

Because a positive correlation exists between intracerebral xanthine concentrations and animal activity (30) and also between plasma concentrations of psychotropic substances and effects on EEG (15), the presented results can be partially explained on a pharmacokinetic basis.

The catechin can be easily considered as responsible for the paradoxical effects of cola extract. The catechin possesses sedative (21) and antihypertensive effects in stressed animals. Moreover, it activates the catecholamine-*O*-methyl-transferase and, as a result, diminishes the concentration of catecholamines (3). Thus, such an action by the catechin could partially antagonize the action of the caffeine present in cola extract, reducing at the same time the caffeine-induced cortical desynchronization.

ACKNOWLEDGEMENTS

The authors thank J. Vion-Dury (URA CNRS 1186, Faculté de Médecine, Marseille) for their collaboration in preparation of this manuscript.

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