Pharmacology Biochemistry & Behavior, Vol. 26, pp. 841–845. Pergamon Journals Ltd., 1987. Printed in the U.S.A. 0091-3057/87 $3.00 + .00

BRIEF COMMUNICATION

Behavioral Effects Resulting From Sub-Chronic Treatment of Rats With Extract of Fresh Stabilized Cola Seeds

G. SCOTTO,* C. MAILLARD,†[1] J. VION-DURY,* G. BALANSARD† AND G. JADOT‡

*Laboratoire de Pharmacologie, Faculté de Médecine
†Laboratoire de Pharmacognosie, Faculté de Pharmacie
‡Laboratoire de Pharmacologie, Faculté de Pharmacie, 27 Boulevard Jean Moulin, 13385 Marseille Cédex 5, France

Received 5 February 1986

SCOTTO, G., C. MAILLARD, J. VION-DURY, G. BALANSARD AND G. JADOT. *Behavioral effects resulting from sub-chronic treatment of rats with extract of fresh stabilized cola seeds.* PHARMACOL BIOCHEM BEHAV **26**(4) 841–845, 1987.—The aim of our study was to compare the effects of a sub-chronic treatment with fresh cola seed extract and pure caffeine in the male rat. The activity tests (open-field) and reactivity (tail-tap, resistance to capture), show that fresh cola has an effect on behavior similar to that of caffeine. However, the effects of cola are more gradual than those of caffeine. Furthermore, cola administration leads to an increase in the fall latency observed during the grasping test. These results suggest that the fresh cola seed has both psychostimulating properties similar to those of caffeine, and an original effect on muscular tonus.

Caffeine Fresh cola seeds Behavior Antifatigue effect Psychostimulant

A certain number of natural substances are known to have a stimulating effect on the behavior of mammals in general and man in particular. One of these, caffeine, has undoubtedly been studied more than the others, from both a psychopharmacological and biochemical standpoint [10]. On the other hand, the cola seed, which is used in therapeutics because of its psychostimulant effects due to the caffeine it contains, has been studied only rarely in the past and results of these previous studies are based on poorly defined experimental procedures [2, 12, 13, 24].

The chemical study of seeds has shown the differences which exist between on the one hand the fresh seed or extract of fresh stabilized cola seeds and on the other hand the dry seed or caffeine [16].

Thus, the pharmacodynamic effects of dried seeds is limited to only the effect of the caffeine [5,17]. On the other hand, the fresh seed or cola extract has a more moderated and longer-lasting effect than caffeine in the light of previous clinical observations [1, 2, 6, 7] and particularly the pharmacokinetic results obtained recently [15]. It has been possible to show that the pharmacokinetic profile and parameters differ significantly when using pure caffeine and fresh seed extract on rats receiving acute or chronic treatment [15]. The lack of experimental work on behavioral effects of the fresh cola seed led us to carry out a survey which could indicate the type of effect this psychostimulant has on behavior. After observing that the effects of fresh cola are more gradual than those of caffeine [2, 12, 13, 24] we carried out a sub-chronic study of the behavioral effects of this product.

METHOD

Plant and Caffeine Material

The atomized extract of stabilized fresh cola seeds [Cola nitida (Vent) A. Chev.] was obtained using the Note method

[1]Requests for reprints should be addressed to Dr. C. Maillard, Laboratoire de Pharmacognosie, Faculté de Pharmacie, 27, Boulevard Jean Moulin, 13385 Marseille Cédex 5, France.

TABLE 1

AVERAGE NUMBER OF SQUARES COVERED IN 5 MINUTES BY THE ANIMALS (n=10) IN EACH GROUP (±S.D.)

	Treatment				After Treatment	
	Days					
Groups	1	4	9	14	18	22
Control	83 (±14.4)	30 (±6.2)	23 (±7.0)	40 (±9.7)	39 (±10.6)	31 (±7.03)
Caffeine	235 (±19.7) §#	124 (±14.1) §	103 (±13.51) §	107 (±10.6) §	73 (±12.58)	51 (±5.5) *
Cola	140 (±8.2) †	85 (±6.67) ¶	87 (±6.23) †	124 (±16.56) †	74 (±11.66)	58 (±4.88) †

Statistical signification: Comparisons: *caffeine/control, †cola/control, ‡caffeine/cola, $p<0.05$; §caffeine/control, ¶cola/control, #caffeine/cola, $p<0.01$.

Average number of squares covered in 5 minutes by the animals (n=10) in each group (±S.D.).

TABLE 2

AVERAGE RESISTANCE TO CAPTURE SCORE (n=10, ±S.D.)

	Treatment				After Treatment	
	Days					
Groups	1	4	9	14	18	22
Control	0.7 (±0.23)	0.7 (±0.23)	0.4 (±0.16)	0.5 (±0.16)	0.7 (±0.27)	0.9 (±0.26)
Caffeine	1.8 (±0.31) §	1.3 (±0.22) *	1.6 (±0.16) §	1.5 (±0.16) §	1.4 (±0.23) #	1 (±0.23)
Cola	1.3 (±0.24)	1.2 (±0.21)	1.4 (±0.16) ¶	1.6 (±0.16) ¶	0.7 (±0.22)	0.5 (±0.23)

Statistical signification: see Table 1.

Average resistance to capture score (n=10, ±S.D.).

[20]. This technique makes it possible to obtain an extract which contains the active substances of the fresh seed with the following components: caffeine (6.2%), theobromine (0.9%), catechine (15%). Caffeine (Carlo Erba) is the reference molecule. Extract and caffeine aqueous solutions are prepared fresh as needed.

Animals

The subjects were 30 adult male Wistar-AF genotoxenic rats, with an average weight of 300±20 g, bred by IFFA CREDO (France). They were housed individually, submitted to a natural dark light cycle and were maintained throughout on food (U.A.R.) and water ad lib.

Method

Substances were administered per os between 9 and 12 a.m. (1 ml at 37°C) for two weeks. The animals were randomly divided into 3 groups of 10: the control group was administered 1 ml of distilled water, the second group 20 $mg \cdot kg^{-1}$ of pure caffeine and the last group 320 $mg \cdot kg^{-1}$ of cola extract (i.e., 20 $mg \cdot kg^{-1}$ of pure caffeine). Behavior was observed on the 1st, 4th, 9th and 14th days of treatment, as well as the 3rd and 7th day after treatment. Behavior tests were carried out one hour after the drugs were administered taking into account the pharmacokinetic results (the maximum concentration of caffeine and cola in the plasma was obtained respectively about 1.5 and 1 hour) [15]. The animals were dealt with at random so as to prevent problems of circadian rhythm.

Statistical Analysis

Experimental values were compared using the Student test after comparing variations with the Snedecor F-test.

TABLE 3

AVERAGE "TAIL-TAP" RESPONSE SCORE (n=10, ±S.D.)

	Treatment				After Treatment	
	Days					
Groups	1	4	9	14	18	22
Control	0.2 (±0.11)	0.2 (±0.11)	0.2 (±0.11)	0.2 (±0.11)	0.3 (±0.14)	0.5 (±0.23)
Caffeine	1.9 (±0.31) ‡§	1 (±0.23) §	0.7 (±0.22) §	1.2 (±0.22) §	1 (±0.29) ‡	1 (±0.24)
Cola	0.7 (±0.14)	0.5 (±0.16)	1.2 (±0.11) ¶	1.6 (±0.23) ¶	0.7 (±0.22)	0.6 (±0.23)

Statistical signification: see Table 1.
Average "tail-tap" response (n=10, ±S.D.).

TABLE 4

AVERAGE LAPSE (IN SECONDS) BETWEEN THE MOMENT THE ANIMAL (n=10) IS SUSPENDED AND HIS FALL TO THE GROUND (±S.D.)

	Treatment				After Treatment	
	Days					
Groups	1	4	9	14	18	22
Control	6 (±0.47)	6.4 (±0.5)	6.1 (±0.52)	4.7 (±0.46)	5.9 (±0.57)	4.8 (±0.51)
Caffeine	6.2 (±0.95)	5.7 (±0.79)	4.5 (±0.64)	4.4 (±0.70) #	5.5 (±0.78)	5.6 (±0.91)
Cola	6.5 (±0.64)	5.4 (±0.70)	6.2 (±0.61)	7.3 (±0.6) ¶	8.3 (±0.59)	7 (±0.89)

Statistical signification: see Table 1.
Average lapse (in seconds) between the moment the animal (n=10) is suspended and his fall to the ground (±S.D.).

Behavioral Tests

Open-field test [26]. This test is used to study the animal's exploratory and locomotor activity in a brightly lit, squared area. During the 5 minute testing time (1) the number of squares crossed and (2) the number of times the animal was erected on back paws presenting an exploratory behavior with sniffing ("rearing test"), were both counted.

Reactivity tests. (a) Resistance to capture test [21]: this test is used to assess, by way of a behavioral scale, the animals's reactivity when handled.

(b) Tail-tap test [21]: this test is used to assess animal reactivity following a tap at the base of the tail.

(c) Grasping test [3]: designed to study the myorelaxing effect of certain psychotropic drugs such as benzodiazepines. The main factor is the lapse of time between the moment the animal is suspended on a wire and the moment it falls to the ground.

RESULTS

Motor Activity Test (Open-Field)

Locomotion (Table 1). During the 15 days of treatment, the average number of squares covered by the rats treated with caffeine or cola extract is significantly greater than that covered by the control group. However, on the first day of experimentation the rats treated with caffeine showed a higher locomotor activity than the rats treated with cola. This difference lessened from the 4th day. On the 9th day the difference was minimal and on the following days the cola group showed a greater motor activity. Once administration ceased, the locomotor activity or the animals treated with caffeine or cola extract remained greater than the control group.

Rearing. There was no great difference between the groups in this respect on the 1st day of treatment. However,

there seems to be a more rapid but no significant decrease in the number of times the control group reared than with the rats from the two other groups.

Reactivity Test

Resistance to capture (Table 2). Animal reactivity remained stable throughout experimentation. From the 1st day, animals treated with caffeine showed a greater resistance to capture than those of the control group. This continued until the 3rd day after treatment was stopped. The cola group showed a later but more significant resistance to capture from the 9th day onwards.

Tail-tap test (Table 3). This test showed development similar to that noted during the resistance to capture test.

Grasping test (Table 4). This test shows the lack of myorelaxation effect with the animals treated with caffeine and the control animals. On the other hand, a notable increase in the latency to fall was observed with the cola group as from day [14]. This increase seemed to continue after treatment was stopped.

DISCUSSION

The oral administration of caffeine led to an increase in exploratory and locomotor activity, and the general reactivity of the animal [10, 25, 26]. The administration of the cola seeds led to effects similar to those noted following treatment with caffeine. This similarity is undoubtedly due to the presence of caffeine in the cola seed. The main difference noted in the effects of the two drugs is, as was suggested by Chevrotier and Vigne [7], in the time taken for the drug to take effect. Whereas the effects of pure caffeine are at a maximum one hour after administration on all the tests from the first day onwards, the effects of the cola extract become equal to those of caffeine only after 9 days of administration. Such differences can be explained by the combination of caffeine and catechin in fresh cola [16]. This combination, present in vivo, clearly modifies the pharmacokinetic parameters of the caffeine contained in the cola extract used [15].

Indeed, during chronic administration of fresh cola, caffeine combined with catechin seems to bind more to the plasmatic proteins than pure caffeine. In this way the free fraction of caffeine is lower when administering cola (47 to 59%) than when administering pure caffeine (65 to 87%). This would tend to suggest that central nervous effects of caffeine are weaker when administering cola than when administering pure caffeine [15]. Nevertheless, repeated cola administration could lead progressively to tissue concentrations of caffeine similar to that obtained by administering pure caffeine. This could take into account the fact that the effect of cola treatment equals that of caffeine treatment starting from the 9th day of experimentation. Such pharmacokinetic characteristics could also take into account the existence of a slightly greater residual effect after cola treatment as opposed to caffeine treatment.

Another difference can be noted concerning the decrease of the effects of the two drugs in the different behavioral tests during treatment. In the open-field test, the reduction of locomotor activities between the first day (as 100%) and respectively the 4th and 9th days of treatment, is less in the Cola group (−40%, −38%) than in caffeine group (−48%, −57%) or control one (−64%, −77%). A similar difference can be noted in tail-tap and grasping tests. This effect might be interpreted as difference in tolerance to caffeine induced by the other compounds of the cola seed. Tolerance to caffeine is known to develop quickly in animal [11] and in man [23], and the delay (4 days) observed in the reduction of locomotor activities in our experiment is compatible with this previous work. The reasons of this apparent difference in caffeine tolerance between caffeine treatment and cola treatment are not clear and perhaps involve central adenosine receptors [4]; yet, pharmacokinetic interactions can't be discarded.

The grasping test made it possible to bring to light an original effect of cola. Indeed, the considerable increase (more than 50%) in the latency to fall after sub-chronic cola administration, tends to indicate that this extract has a specific effect on muscular tonus even if, in man and rat, caffeine was demonstrated to have an antifatigue effect [8]. This original effect of cola seed could be similar to the antifatigue effect previously described by Barr in man [2]: this author noted an increase in the time taken to implement muscular effore more than an increase in force developed, under cola treatment. Other tests should be carried out in order to more clearly define the way in which cola exhibits an antifatigue effect; but it would seem, after this experimentation, that cola is a psychostimulant with some properties exhibited resembling those of caffeine.

Because of the caffeine it contains, cola could act by competitive inhibition of the link between adenosine and its cerebral receptors [28]. Nevertheless, the richness of the fresh cola seed in catechin derivates and amino acids [15] could explain the more specific and original effect on muscular contraction, without it actually being possible to say whether or not this takes place on a central or peripheral level.

In this point of view, metabolism [9] and physiological activity of catechin and other bioflavonoids on several targets are well known: catechin induces a reduction of free radicals production [27], and has a protective effect on blood vessels [18] and hepatocytes [22].

A recent paper has suggested that bioflavonoids might be responsible for the antihypertensive effects of decaffeinated tea [14]. When it is assumed that open-field test is a stressing design, the differences observed in locomotor and exploratory activities between cola and caffeine treatments might also be related both to the increase sympathoadrenal activity under the influence of caffeine [29] and to the decrease of this same function due to the catechins contained in cola [14]. This "beta blocking like" effect of catechins and the possible action of these compounds on the activity of cola-treated animals would be explained by reports of the sedative effects of catechins [19]. Some results on EEG activity after a cola treatment (Vion-Dury and Coll, submitted) are also in agreement with this hypothesis.

In conclusion, it seems that the fresh cola extract presents an original and complex activity spectrum which is probably related to both presence of caffeine and catechins and it seems that during cola treatment the catechins act adversely against caffeine action by indirect (pharmacokinetic) or direct (central action, changes in tolerance phenomenons) pathways. But the antifatigue action seems more specific and could represent an interesting development of pharmacologic properties of cola. News studies are under way in our laboratory in order to determine the nervous and muscular effects of catechins.

ACKNOWLEDGEMENTS

The authors wish to express their gratitude to the LAPHAL Laboratory for having helped them in the course of this study.

REFERENCES

1. Allers, R. and E. Freund. Zur Kenntnis der Wirkung von Kola. Präparaten. *Z Gesamte Exp Med* **49**: 644–648, 1926.
2. Barr, G. W. The physiological action of Kola. *Ther Gazette* **20**: 221–226, 1896.
3. Boissier, J. R. and P. Simon. Influence de la caféine sur le comportement en situation libre de la souris. *Arch Int Pharmacodyn Ther* **166**: 362–369, 1967.
4. Boulenger, J. P., J. Patel, R. M. Post, S. Marma and P. J. Marangas. Effets de l'administration prolongée de caféine sur les récepteurs cérébraux de l'adénosine et des benzodiazèpines. *Acta Pharmacol Biol Clin* **2**: 151–154, 1983.
5. Chevalier, A. and E. Perrot. *Les Kolatiers et les Noix de Kola*. Paris, France: A. Chalamel, 1911, pp. 219–284.
6. Chevalier, J. and A. Goris. Action pharmacodynamique de la Kolatine. *C R Acad Sci [D] (Paris)* **145**: 354–355, 1907.
7. Chevrotier, J. and P. Vigne. Sur la noix de Kola fraîche. *Bull Soc Pharmacol* **13**: 620–624, 1906.
8. Costill, D. L., G. P. Dalsky and W. J. Fink. Effects of caffeine ingestion on metabolism and exercise performance. *Med Sci Sports* **10**: 155–158, 1978.
9. Das, N. P. Studies on flavonoid metabolism. *Biochem Pharmacol* **20**: 3435–3445, 1971.
10. Dews, P. B. *Caffeine: Perspectives From Recent Research*. Berlin: Springer-Verlag, 1984.
11. Gilbert, R. M. Caffeine as a drug abuse. *Res Adv Alcohol Drug Probl* **3**: 49–176, 1976.
12. Heckel, E. Sur l'action du kola à propos des effets de la caféine. *Bull Gen Ther Med* **118**: 345–351, 1890.
13. Heckel, E. Expériences comparées entre l'action du kola et de la caféine. *Marseille Med* **27**: 586–598, 649–665, 1890.
14. Henry, J. P. and P. Stephens-Larson. Reduction of chronic psychosocial hypertension in mice by decaffeinated tea. *Hypertension* **6**: 437–444, 1984.
15. Maillard, C. Contribution à l'étude de la graine de Cola nitida (Vent) A. Chevalier. Thèse Pharm. Marseille, 1985.
16. Maillard, C., A. Babadjamian, G. Balansard, B. Ollivier and D. Bamba. Study of caffein-catechin association in lyophilized fresh seeds and in stabilized extract of Cola nitida. *Planta Med* **6**: 515–517, 1985.
17. Mascre, M. and R. Paris. Fruits et graines d'Outremer, utilisés en thérapeutique. *Fruits d'Outremer* **1**: 226–230, 1946.
18. Michaud, J. and J. Masquelier. Quelques aspects nouveaux de la connaissance des tanins catéchiques leurs relations avec la vitamine C_2 (P). *Prod Probl Pharmacol* **28**: 499–520, 1973.
19. Murari, R., S. Natrajan and T. R. Seshadri. Barbiturate induced sleeping time in mice by tea polyphenols. *Curr Sci* **42**: 540–541, 1973.
20. Note, D. P., G. Balansard, C. Maillard and G. A. Rovira. Procédé de préparation d'un extrait atomisé de graines fraîches stabilisées de Cola. I.N.P.I., Paris No. 8513067, B.B. 32888, September 1985.
21. Pinel, J. P. J., D. Treit and L. I. Rovner. Temporal lobe aggression in rats. *Science* **197**: 1088–1090, 1977.
22. Reutter, W., B. Hassels and R. Lesch. Induction et prévention de l'hépatite à la galactosamine. *New Trends in the Therapy of Liver Diseases*. International Symposium, Tirrenia, 1974, pp. 121–127.
23. Robertson, D., D. Wade, R. Workman and R. L. Woosley. Tolerance to the humoral and hemodynamic effects caffeine in man. *J Clin Invest* **67**: 111–117, 1981.
24. Rodet, P. De l'action comparée de la kola et de la caféine sur la nutrition. *Bull Soc Med Prat (Paris)*, 468–478, 1891.
25. Simon, P. Contribution à l'étude chez l'animal de l'action psychotrope de la caféine. Thèse Med., Paris, 1965.
26. Sinton, C. Investigation neurocomportementale de l'action de la caféine chez le mammifère. These Med., Lyon, 1981.
27. Slater, T. F. and M. N. Eakins. Interactions of cianidol with free radical generating systems. *New Trends in the Therapy of Liver Diseases*. International Symposium, Tirrenia, 1974, pp. 84–89.
28. Snyder, S. H., J. J. Katims, Z. Annau, R. F. Bruns and J. W. Daly. Adenosine receptors and behavioral actions of methylxanthines. *Proc Natl Acad Sci USA* **78**: 3260–3265, 1981.
29. Spindel, E. R., R. J. Wurtman, D. B. Carr, L. Conlay, L. Griffith and M. A. Arnold. Neuroendocrine effects of caffeine in normal subjects. *Clin Pharmacol Ther* **36**: 402–407, 1984.