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BRIEF COMMUNICATION

Differential Biochemical Mechanisms Mediate Locomotor Stimulation Effects by Caffeine and Nicotine in Rats

EMINY H. Y. LEE, MAY J. TSAI, YU P. TANG AND CHOK Y. CHAI

Institute of Biomedical Sciences, Academia Sinica, Taipei (11529), Taiwan, The Republic of China

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LEE, E. H. Y., M. J. TSAI, Y. P. TANG AND C. Y. CHAI. *Differential biochemical mechanisms mediate locomotor stimulation effects by caffeine and nicotine in rats.* PHARMACOL BIOCHEM BEHAV **26**(2) 427–430, 1987.—Effects of caffeine and the interactive effects of caffeine and nicotine on locomotor activity in rats were examined in the present study. Other than confirming previous reports that both drugs enhanced locomotion, we have also found that their effects on activity were additive. Meanwhile, results of various biochemical measures have revealed that at the minimum effective doses of caffeine and nicotine which facilitated locomotor activity, only one biochemical system was preferentially influenced by either drug alone. The most significant findings were that caffeine stimulated the release of catecholamines and nicotine decreased the concentrations of tyrosine and tryptophan in brain. The combined effects of caffeine and nicotine on these brain amines were not different from those of each drug alone. Together with the report that caffeine and nicotine had differential actions on different activity measures, the present results support the hypothesis that caffeine and nicotine affect locomotor activity via different neurochemical mechanisms.

Caffeine Nicotine Locomotor activity Catecholamine Cyclic AMP Amino acid precursors High performance liquid chromatography

BOTH caffeine and nicotine are known to be psychoactive compounds in the central nervous system. Behaviorally, both drugs have been demonstrated to stimulate animals' locomotor activity in different behavioral paradigms [8, 10, 12, 19]. Recently, Meliska and Loke [12] have reported that although both caffeine and nicotine increased ambulation of rats in an open field, nicotine depressed wheelrunning activity while caffeine increased it. In addition, caffeine also increased the frequencies of rearing and holepoke in an open field, while nicotine did not alter these exploratory measures significantly [10,12]. These results suggest that caffeine and nicotine probably affect locomotor activity via different neuropharmacological mechanisms.

Biochemically, accumlative evidence has revealed that caffeine stimulated the release of catecholamine (CA) [norepinephrine (NE) and dopamine (DA)] [1,19], antagonized the effects of adenosine in brain [3,16], and inhibited the activity of phosphodiesterase, an enzyme which breaks down cyclic adenosine-3′,5′-monophosphate (cyclic AMP) [14]. Nicotine was shown to interact with a variety of neurotransmitter systems [4, 11, 17]. More recently, we have found that nicotine also decreased tyrosine and tryptophan concentrations in several brain regions and these effects were most significantly correlated with the stimulatory effect on locomotion produced by nicotine [10]. If caffeine and nicotine do affect activity through different neurochemical mechanisms, as suggested by the above behavioral observations, their effects on locomotor activity should be additive when administered together. The present behavioral and biochemical results support this hypothesis.

METHOD

Sevently-four experimentally naive male Sprague-Dawley rats (200–250 g) from the Animal Resource Center, National Taiwan University, Taiwan were used. They were housed in groups of three rats in a temperature-regulated (25±2°C) room on a 12/12 hour light/dark cycle with water and Purina Rat Chow available ad lib. The animals were kept for at least seven days before experimentation.

Caffeine (anhydrous powder) and nicotine (free base, Sigma) were dissolved in 0.9% isotonic saline and diluted in series.

Locomotor activity was monitored in an activity chamber as described previously [10]. A 4×4 perpendicular array of infrared photobeams 5/8 in. above the floor were used to localize the animal's floor position. The behavioral apparatus was connected to a control unit to check the status of the beams and circuits in each chamber. Any change during the

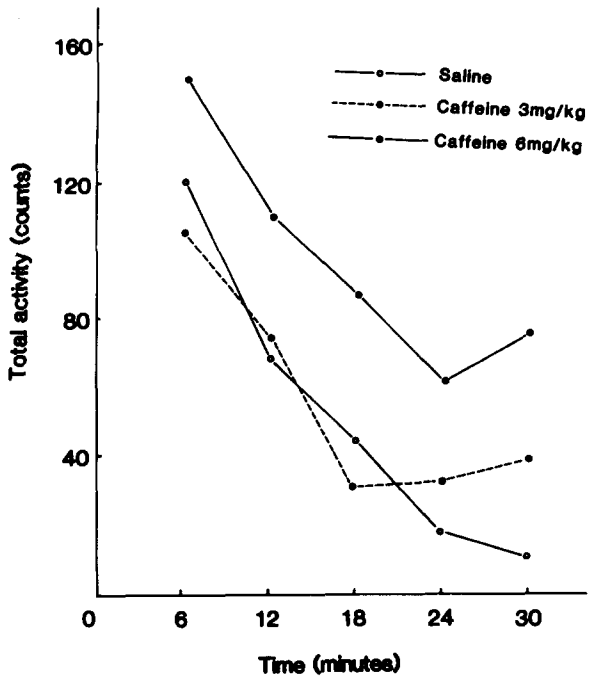

FIG. 1. Dose-response effects of caffeine on locomotor activity. Only the 6 mg/kg dose of caffeine significantly increased locomotion.

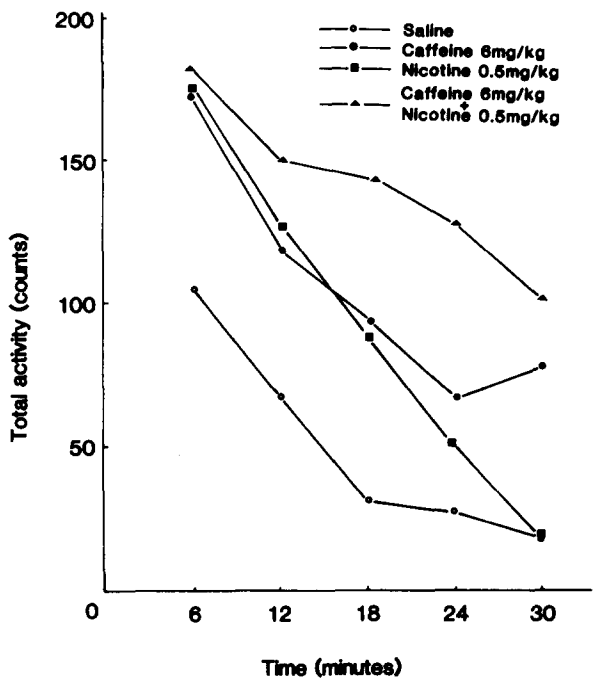

FIG. 2. Interactive effects of caffeine and nicotine on locomotor activity. Additive effect was observed when caffeine and nicotine were administered together. The activity level of the caffeine plus nicotine group was significantly higher than that of either the caffeine or the nicotine group alone.

test was automatically taken by the control unit and displayed on the printer.

Animals were brought to the laboratory at least one hour before testing. For experiment 1, thirty animals were randomly assigned to three groups of ten rats each and were given saline, 3 mg/kg or 6 mg/kg caffeine. The appropriate dose was then used for the following interaction study. For experiment 2, animals were divided to four groups of eight rats each and received injections of saline, 6 mg/kg caffeine (the minimum effective dose found in experiment 1), 0.5 mg/kg nicotine (the minimum effective dose found previously [10]), or combination of caffeine and nicotine at the same doses used above. For experiment 3, animals were given either saline (n=6) or 0.5 mg/kg nicotine (n=6) to examine the effects of nicotine on regional cyclic AMP. Caffeine was given intraperitoneally while nicotine was injected subcutaneously. Half of the animals in control groups received subcutaneous and the other half received intraperitoneal injections of saline. The injection volume was 1 ml/kg. Experiments started 1 min after injections. The number of cumulative response was recorded every 6 min for a total of 30 min. All behavioral testing was performed in the light phase of the diurnal rhythm. Animals were killed by decapitation immediately after testing. Their brains were taken out in 90 sec, dissected on ice and frozen at −80°C until biochemical assays.

Monoamines and their precursors were estimated using high performance liquid chromatography (HPLC) with fluorescence detection according to the method of Peat and Gibb [13] with some modification as described in detail elsewhere [10]. Briefly, tissue was weighed while still frozen and homogenized in 10 vol. of 0.1 M perchloric acid containing 4 mM sodium metabisulfite. The homogenate was then centrifuged at 15,000 rpm for 15 min using a refrigerated centrifuge, and the clear supernatant was injected directly into the chromatographic system.

Cyclic AMP was assayed using HPLC with ultraviolet detection according to the method of Krstulovic *et al.* [9] with some modification. Because tissue extraction was the same for monoamines and cyclic AMP, the supernatant from the same animal following monoamine assay was directly injected into the system for cyclic AMP assay.

The behavioral data were analyzed using a two-way mixed design analysis of variance (ANOVA) with group as the between-subjects factor and time as the repeated measure. Specific comparisons between each treatment group and a common control group at different time points were made with Dunnett's method and comparison between experimental groups were made with Newman-Keuls method. The biochemical data were analyzed using the Student's *t*-test [20].

RESULTS

Figure 1 illustrates the dose-response effects of caffeine on locomotor activity. The mixed-design ANOVA revealed a significant overall effect of caffeine, $F(2,24)=19.45$, $p<0.01$, but not a significant caffeine-by-time interaction effect on locomotion, $F(8,96)=0.78$, n.s. Further main effect analyses indicated that only 6 mg/kg caffeine markedly augmented activity measure, $tD(3,24)=2.72$, $p<0.05$.

Effects of caffeine (6 mg/kg), nicotine (0.5 mg/kg) and

TABLE 1

EFFECTS OF CAFFEINE AND NICOTINE ON TYROSINE, TRYPTOPHAN, MONOAMINE AND CYCLIC AMP LEVELS

Treatment	N	Tyr		Trp		NE		DA		Cyclic AMP	
		Hippo	Hypo	ST	Hippo	Hypo	ST	Hippo	Hypo	ST	Hippo	Hypo	ST
Saline	8	11047±573	11670±642	12166±458	4871±161	3491±113	4902±121	773± 50	2438±101	10526±421	144± 7	38±3	127± 7
Caffeine (6 mg/kg)	8	9540±75	10677±594	12013±800	4399±132	3241±161	5229±197	399±107†	2690±111	9031±768*	152±10	35±2	116± 7
Nicotine (0.5 mg/kg)	8	8589±666†	10634±310	10439±437*	4262±109*	2899±130†	4245±220*	—	—	—	135± 6	44±4	118±14
Caffeine (6 mg/kg) + Nicotine (0.5 mg/kg)	8	8897±354†	10880±261	10505±318*	4337±401*	3082±159*	5193±213	817±126	2532±212	10027±155	120± 5	34±2	125±10

Hippo: Hippocampus, Hypo: Hypothalamus, ST: Striatum.
*$p<0.05$ and †$p<0.01$. Statistical significance was evaluated using Student's t-test.
The data are expressed as ng/g tissue for catecholamines and μg/g tissue for cyclic AMP. Values are means±SEM and are not corrected for recovery.

combination of these two drugs on locomotor activity are shown in Fig. 2. The mixed design ANOVA revealed a significant main effect of caffeine and nicotine on locomotion, respectively, $F(4,32)=2.77$, $p<0.05$, and, $F(4,32)=2.59$, $p<0.05$. Combination of caffeine and nicotine further augmented locomotor activity [$F(4,32)=3.78$, $p<0.01$, NK=44.82, $p<0.01$ when compared with caffeine group and NK=47.64, $p<0.05$ when compared with nicotine group]. Further analyses revealed that their effects were not additive for the first 6 min of behavioral testing.

The major biochemical effects of caffeine and nicotine are summarized in Table 1. Table 1 revealed that caffeine at 6 mg/kg significantly decreased NE concentration in the hippocampus, $tD(4,28)=3.20$, $p<0.01$, reduced DA level in the hypothalamus, $tD(4,28)=2.25$, $p<0.05$, and striatum, $tD(4,28)=2.23$, $p<0.05$. Cyclic AMP, tyrosine and tryptophan levels were not markedly changed by the same dose of caffeine in these regions (all $p>0.05$, n.s.).

As also indicated in this table, nicotine at 0.5 mg/kg significantly decreased tyrosine and tryptophan concentrations in the striatum, $tD(4,28)=2.35$, $p<0.05$ and $tD(4,28)=2.52$, $p<0.05$, and hippocampus, $tD(4,28)=3.19$, $p<0.01$ and $tD(4,28)=2.20$, $p<0.05$. Tryptophan level in the hypothalamus was also reduced by the same dose of nicotine, $tD(4,28)=3.12$, $p<0.01$. Cyclic AMP was not significantly altered by nicotine in all three regions examined (all $p>0.05$, n.s.). In addition, effects of combination of these two drugs were not significantly different from that of each drug alone on all measures.

DISCUSSION

The present results extend previous studies relating the locomotor stimulating effects to the biochemical actions of caffeine and nicotine. Other than confirming the majority of previous findings that both caffeine and nicotine enhanced locomotion [8, 10, 12, 19], we have further demonstrated that effects of these two drugs on activity are additive. In addition, caffeine also increased animals' rearing and hole-poke frequencies in an open field (unpublished observations), while nicotine did not alter these exploratory measures significantly [10]. Together with the report that caffeine facilitated wheelrunning activity while nicotine inhibited it in rats [12], the present results support the hypothesis that caffeine and nicotine probably augment locomotor activity through different mechanisms.

Biochemical investigations have demonstrated that nicotine stimulated central cholinergic receptors [2,18] and excited central CA neurons [11,17]. We have found that nicotine also decreased brain tyrosine and tryptophan concentrations, and the reduction of these amino acid precursors is significantly correlated with the augmentation of locomotor activity [10]. However, nicotine at the same dose (0.5 mg/kg) did not produce a consistent and significant influence on brain monoamine and cyclic AMP levels. Similarly, caffeine was shown to stimulate central CA release [1,19], and the catecholaminergic blocking agents antagonized its effects on locomotion [5,6]. Another major action of caffeine was inhibition of the breakdown of cyclic AMP by inactivating the enzyme phosphodiesterase [14]. In the present study, we measured both CA and cyclic AMP levels and have demonstrated that caffeine at 6 mg/kg selectively decreased CA level without significantly altering cyclic AMP content in several brain regions. The amino acid precursors

tyrosine and tryptophan in the few regions examined were not markedly altered by caffeine either. These results are incongruent with the findings of Fernstrom *et al.* and Schlooberg *et al.* that caffeine increased brain tryptophan, but without affecting DA and NE [7,15]. However, the dose of caffeine used in their studies was much higher (100 mg/kg) than the dose used here. Results from our study are consistent with most literatures that caffeine primarily acts on central CA systems. The reduction in cellular CA, probably as a consequence of neuron excitation, could be attributed to an increased release and utilization of CA. Lack of an effect of caffeine on cyclic AMP is also consistent with the report that concentrations of caffeine required to produce activity changes via cyclic AMP enhancement greatly exceed doses which facilitate activity [16].

Although accumulative evidence has demonstrated that both caffeine and nicotine interacted with a variety of neurochemical and biochemical systems, most studies used rather high doses of the drugs which lose the specificity of these compounds in the central nervous system. In the present study, we used the minimum effective doses of caffeine and nicotine to examine their behavioral and neurochemical correlates. Results support our hypothesis that the stimulatory effects of caffeine and nicotine on locomotor activity are additive through different mechanisms. Caffeine enhanced locomotion primarily through stimulating central CA release and nicotine augmented locomotion through its actions on brain tyrosine and tryptophan.

Both caffeine and nicotine are widely used psychoactive compounds in humans. The present study using threshold doses of both drugs which preferentially influence only one biochemical system may help to further elucidate the mechanisms of these compounds in the central nervous system.

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REFERENCES

1. Berkowitz, B. A., J. H. Tarver and S. Spector. Release of norepinephrine in the central nervous system by theophylline and caffeine. *Eur J Pharmacol* **10:** 64–71, 1970.
2. Clarke, P. B. S. and R. Kumar. The effects of nicotine on locomotor activity in non-tolerant and tolerant rats. *Br J Pharmacol* **78:** 329–337, 1983.
3. Daly, J. W., R. F. Bruns and S. H. Snyder. Adenosine receptors in the central nervous system: Relationship to the central actions of methylxanthines. *Life Sci* **28:** 2083–2097, 1981.
4. Engberg, G. and T. H. Svenssen. Pharmacological analysis of a cholinergic receptor mediated regulation of brain norepinephrine neurons. *J Neural Transm* **49:** 137–150, 1980.
5. Estler, C. J. Effects of α- and β-adrenergic blocking agents and parachlorophenylalanine on morphine- and caffeine-stimulated locomotor activity of mice. *Psychopharmacologia* **28:** 261–268, 1973.
6. Estler, C. J. Influence of pimozide on the locomotor hyperactivity produced by caffeine. *J Pharm Pharmacol* **31:** 126–127, 1979.
7. Fernstrom, M. H., C. W. Bazil. and J. D. Fernstrom. Caffeine injection raises brain tryptophan level, but does not stimulate the rate of serotonin synthesis in rat brain. *Life Sci* **35:** 1241–1247, 1984.
8. Hughes, R. N. and A. M. Greig. Effects of caffeine, methamphetamine and methylphenidate on reaction to novelty and activity in rats. *Neuropharmacology* **15:** 673–676, 1976.
9. Krstulovic, A. M., R. A. Hartwick and P. R. Brown. Reversed-phase liquid chromatographic separation of 3′,5′-cyclic ribonucleotides. *Clin Chem* **25:** 235–241, 1979.
10. Lee, E. H. Y. Effects of nicotine on exploratory behavior in rats: correlation with regional brain monoamine levels. *Behav Brain Res* **17:** 59–66, 1985.
11. Lichtensteiger, W., F. Hefti, D. Felix, T. Huwyler, E. Melamed and M. Schlump. Stimulation of nigrostriatal dopamine neurons by nicotine. *Neuropharmacology* **21:** 963–968, 1982.
12. Meliska, C. J. and W. H. Loke. Caffeine and nicotine: Differential effects on ambulation, rearing and wheelrunning. *Pharmacol Biochem Behav* **21:** 871–875, 1984.
13. Peat, M. A. and J. W. Gibb. High performance liquid chromatographic determination of indoleamines, dopamine and norepinephrine in rat brain with fluorimetric detection. *Anal Biochem* **128:** 275–280, 1983.
14. Piafsky, K. H. and R. I. Ogilvie. Drug therapy: dosage of theophylline in bronchial asthma. *N Engl J Med* **299:** 1218–1221, 1975.
15. Schlosberg, A. J., J. D. Fernstrom, M. C. Kopczynski, B. M. Cusack and M. A. Gillis. Acute effects of caffeine injections on neutral amino acids and brain monoamine levels in rats I. *Life Sci* **29:** 173–183, 1981.
16. Snyder, S. H. Adenosine receptors and the actions of methylxanthines. *Trends Neurosci* **4:** 242–244, 1981.
17. Svenssen, T. H. and G. Engberg. Effect of nicotine on single cell activity in the noradrenergic locus coeruleus. *Acta Physiol Scand [Suppl]* **479:** 31–34, 1980.
18. Volle, R. L. and G. B. Koelle. Ganglionic stimulating and blocking agents. In: *The Pharmacological Basis of Therapeutics,* 6th edition, edited by L. S. Goodman and A. Gilman. New York: Macmillan Publishing, 1980.
19. Waldeck, B. Effect of caffeine on locomotor activity and central catecholamine mechanisms: A study with special reference to drug interaction. *Acta Pharmacol Toxicol (Copenh)* **36:** Suppl 4, 1–23, 1975.
20. Winer, B. *Statistical Principles in Experimental Design.* New York: McGraw-Hill, 1971.