J. Pharm. Pharmacol. 1984, 36: 458–460
Received November 25, 1983

© 1984 J. Pharm. Pharmacol.

# Differential effects of caffeine on dihydroxyphenylacetic acid concentrations in various rat brain dopaminergic structures

S GOVONI*, V. V. PETKOV†, O. MONTEFUSCO, C. MISSALE, F. BATTAINI, P. F. SPANO AND M. TRABUCCHI

*Department of Pharmacology and Therapeutics, University of Brescia, Italy; †Institute of Physiology, Bulgarian Academy of Science, Sofia, Bulgaria*

The behavioural and neurochemical effects of caffeine were examined in rats. The intraperitoneal administration of different doses of caffeine significantly decreased DOPAC concentrations in striatum, hypothalamus and frontal cortex, but increased them in nucleus accumbens. These observations suggest that the effects of caffeine on the central nervous system (cns) are at least partially mediated through an interaction with the dopaminergic system.

In laboratory animals caffeine produces behavioural activation and increases startle response (Ward et al 1981), stimulates locomotor activity (Snyder et al 1981b), and potentiates amphetamine- and apomorphine-induced stereotyped behaviour (Klawans et al 1974). Caffeine treatment may also induce a quasi-morphine abstinence syndrome (Francis et al 1975; Lloyd & Stone 1981; Collier et al 1981) and a self-mutilating behaviour (Lloyd & Stone 1981).

Neurochemical studies in-vivo and in-vitro have shown that methylxanthines increase central noradrenaline turnover (Karasawa et al 1976; Waldeck 1971), decrease 5-hydroxytryptamine turnover (Corrodi et al 1972), increase or decrease dopamine turnover (Waldeck 1971; Corrodi et al 1972), release brain catecholamines (Berkowitz et al 1970), inhibit phosphodiesterase activity with resultant accumulation of cyclic adenosine monophosphate (cAMP) and act as competitive antagonists of adenosine receptors (Bruns et al 1980; Williams & Risley 1980; Schwabe & Trost 1980; Snyder et al 1981a, b; Daly et al 1981; Murphy & Snyder 1982). The concentration of methylxanthines required to produce some of the biochemical changes observed is substantially greater than the behaviourally active concentration in the cns (Snyder et al 1981b) and some doubt has arisen as to the possible significance of these changes.

Although caffeine-induced stimulation is a complex phenomenon in which several neurotransmitters are probably involved, a modulatory action of the adenosine system on dopaminergic mechanisms is supported by the fact that theophylline and caffeine potentiate the stereotyped behaviour induced by apomorphine and amphetamine (Klawans et al 1974) and rotational response to apomorphine in rats with lesions of the substantia nigra (Fuxe & Ungerstedt 1974). This view was further stressed in the recent work of Green et al (1982), showing an inhibitory action of an intrastriatal injection of 5′-*N*-ethylcarboxamide-adenosine, an adenosine agonist, on striatal dopaminergic function.

However, although contrasting results have been reported on the effect of caffeine on dopaminergic transmission, these neurochemical studies were on the whole brain. We have examined the effect of acute caffeine administration on dopaminergic transmission in more detail by determining dihydroxyphenylacetic acid (DOPAC) and dopamine (DA) concentrations in the rat striatum, nucleus accumbens, hypothalamus and frontal cortex.

## MATERIALS AND METHODS

Male-Sprague-Dawley rats (Charles River, Calco, Italy) 170–200 g were housed in conditions of constant temperature (20 °C) and humidity with a daily light cycle from 08.00 am to 08.00 pm. The rats had free access to food and water until the time of experiments.

Caffeine was dissolved in 0·9% NaCl and administered in doses of 10, 20, 40 and 100 mg $kg^{-1}$ i.p.

Locomotor activity was recorded for 105 min using an electronic activity cage (Animex, LKB, Sweden). The animals were placed in groups of three in the activity cage and the total number of movements recorded. The tests were all begun 1 h after the end of the 12 h light phase of the light dark cycle. For neurochemical studies the animals were decapitated,

* Correspondence.

the brains removed and the striatum, nucleus, accumbens, hypothalamus and frontal cortex rapidly dissected according to Glowinski & Iversen (1966) quickly frozen on dry ice and kept at −20 °C overnight before biochemical assay. DOPAC concentrations were determined according to the radioenzymatic micromethod of Argiolas et al (1977). DA and DOPAC concentrations in the samples were measured by hplc using a Perkin Elmer Series 2 liquid chromatograph with an electrochemical detector and glassy carbon amperometric detector (TL-5 Bioanalytical Systems) and a reverse phase column (Bio Sil ODS10 250 mm × 4 mm, Bio Rad), equipped with guard column (micro guard ODS-10, Bio Rad). The mobile phase consisted of 0·1 M $Na_2HPO_4$ and 0·1 M citric acid, pH 5·0, EDTA 50 μM, sodium octyl sulphate 30 mg litre$^{-1}$ and methanol 5%, filtered, degassed and pumped at room temperature at a flow rate of 1·5 ml min$^{-1}$ with a potential 0·5 V verus Ag/AgCl reference electrode and a sensitivity set at 1 nA V$^{-1}$.

Catechol compounds were extracted and calculated according to Felice et al (1978). Under our standard conditions, the retention times of authentic and extracted catechols were identical and were: DOPAC 4·0, dihydroxybenzylamine 5·0, and DA 7·6 min, repectively. Protein content was measured by the method of Lowry et al (1951). The results are expressed as mean ± s.d. Statistical significance was calculated by using the Dunnet two-tailed *t*-test.

## RESULTS

The administration of different caffeine doses (10, 20, 40 and 100 mg kg$^{-1}$ i.p.) produced a bell-shaped dose-response curve with an increase of locomotor activity that peaked at 40 mg kg$^{-1}$, but no effect or hypomotility was observed with the highest dose (100 mg kg$^{-1}$).

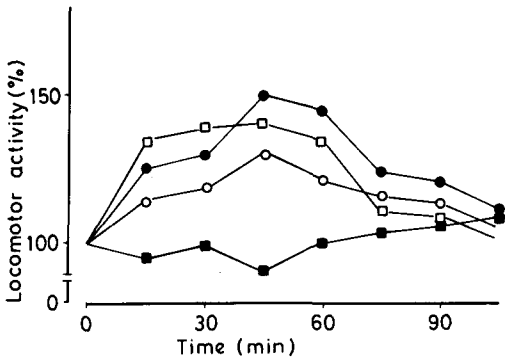


FIG. 1. Time course of the effect of four different caffeine doses (10 ○, 20 □, 40 ● and 100 ■ mg kg$^{-1}$ i.p.) on locomotor activity in the rat. The data are expressed as % of the basal activity. Each point represents the mean of the values obtained with 10–12 animals.

The increase of locomotor activity was maximum between 20 and 45 min after drug treatment, with a return to basal values after 75 min (Fig. 1). The effect of caffeine injections at different doses on DOPAC concentrations in striatum, nucleus accumbens, hypothalamus and frontal cortex is shown in Fig. 2.

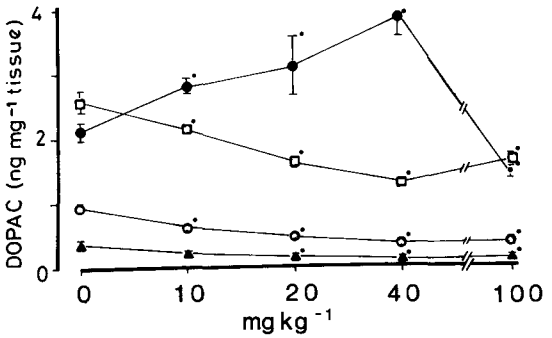


FIG. 2. Dose-response curve of caffeine on the DOPAC concentration in various rat brain regions. *$P < 0·01$ in comparison with DOPAC levels in control animals. Each value represents the mean ± s.d. of three experiments using 5 animals per group. The drug was injected intraperitoneally 20 min before the death of the animals. □ Striatum, ● N. accumbens, ○ Hypothalamus, ▲ Frontal cortex.

Caffeine decreased DOPAC concentrations in a dose-dependent manner in all the structures investigated except the nucleus acumbens where, in contrast, there was an increase. In striatum, hypothalamus and frontal cortex the caffeine effect was maximal at 40 mg kg$^{-1}$, there being no further decrease in DOPAC content with higher doses. In the nucleus accumbens the effect was biphasic with the greatest increase in DOPAC concentration occurring after the 40 mg kg$^{-1}$ dose and a decrease occurring after 100 mg kg$^{-1}$. Both the maximal decrease in DOPAC concentration in striatum, hypothalamus and frontal cortex and the maximal increase in nucleus accumbens occurred 20 min after drug administration (data not shown). In contrast, caffeine induced a dose-dependent increase in dopamine concentration both in striatum and nucleus accumbens (Table 1).

## DISCUSSION

The observed changes in DOPAC concentrations in striatum, nucleus accumbens, hypothalamus and frontal cortex extend previous findings with whole brain extracts that indicated an altered dopamine turnover (Waldeck 1971; Corrodi et al 1972) and then confirm the hypothesis of caffeine action on dopaminergic transmission.

Table 1. Effects of caffeine administration on dopamine (DA, ng $mg^{-1}$ tissue) content in rat striatum and nucleus accumbens.

| | Striatum | N. accumbens |
|---|---|---|
| Saline | 9·35 ± 0·40 | 1·03 ± 0·04 |
| Caffeine 10 mg $kg^{-1}$ | 10·75 ± 0·47* | 1·25 ± 0·05* |
| Caffeine 20 mg $kg^{-1}$ | 11·68 ± 0·60* | 1·42 ± 0·06* |
| Caffeine 40 mg $kg^{-1}$ | 12·06 ± 0·58* | 2·64 ± 0·08* |
| Caffeine 100 mg $kg^{-1}$ | 13·93 ± 0·07* | 2·26 ± 0·06* |

* $P < 0·01$ versus saline treated.
Values are m ± s.d. of at least 5 rats for each group.

Nevertheless, the effect of caffeine is complex, resulting in changes in the striatum, frontal cortex and hypothalamus, where a dose-related decrease of DOPAC concentrations was found, while in nucleus accumbens DOPAC concentrations were increased and a bell-shaped dose-response curve observed.

These opposing trends in different areas may partially account for the contrasting results reported in previous whole brain studies (Waldeck 1971; Corrodi et al 1972).

Most interestingly, DOPAC changes in the nucleus accumbens are reminiscent of the time and dose relationships of the activity changes elicited by caffeine treatment according to the observation of Ward et al (1981) and our own results (Fig. 1).

However, caffeine does not seem to interact directly with dopaminergic receptors in binding studies (Govoni et al 1983). The caffeine-induced changes in DOPAC concentrations, together with a lack of a demonstrable direct action on dopaminergic receptors, may indicate that the behavioural and neurochemical effects involving dopaminergic transmission are predominantly due to a transynaptic mechanism mediated through other neuronal systems. In particular, the observed ability of caffeine to displace specific ligands from adenosine receptors may suggest that the changes in dopaminergic transmission are linked to antagonism of the adenosine system. This view is strengthened by the fact that the dose of caffeine necessary to induce significant behavioural changes gives rise to a sufficient quantity of the xanthine in the brain to interact with central adenosine receptors (Snyder et al 1981b).

Green et al (1982) have provided evidence of an inhibitory regulation of dopaminergic transmission by adenosine-2 receptor (or $R_a$ type, mediating cAMP accumulation) agonists. It is possible that caffeine exerts its stimulatory action by facilitating dopaminergic-mediated behaviour through antagonism of adenosine-mediated inhibitory activity. If the effect of caffeine on dopaminergic transmission is mediated through an action on adenosine receptors, the opposite action of caffeine on DOPAC concentrations in the nucleus accumbens in relation to the other brain areas examined may be due to a different neuronal organization in this area. However, to our knowledge no data are available on adenosine receptor presence and location in the nucleus accumbens. The data presented suggest that caffeine stimulatory effects are at least partially mediated through an interaction with the dopaminergic system.

## REFERENCES


Argiolas, A., Fadda, F., Stefanini, E., Gessa, G. L. (1977) J. Neurochem. 29: 599–601

Berkowitz, B. A., Tarver, J. R., Spector, S. (1970) Eur. J. Pharmacol. 10: 64–71

Bruns, R. F., Daly, J. W., Snyder, S. H. (1980) Proc. Natl. Acad. Sci. (USA) 77: 5547–5551

Collier, H. O. J., Cuthbert, N. J., Francis, D. (1981) Fed. Proc. Fed Am Soc. Exp. Biol. 40: 1513–1518

Corrodi, H., Fuxe, K., Jonsson, G. (1972) J. Pharm. Pharmacol. 24: 155–158

Daly, J. W., Bruns, R. F., Snyder, S. H. (1981) Life Sci. 28: 2083–2097

Felice, L. J., Felice, J. D., Kissinger, P. T. (1978) J. Neurochem. 31: 1461–1465

Francis, D. L., Roy, A. C., Collier, H. O. J. (1975) Life Sci. 16: 1901–1906

Fuxe, K., Ungerstedt, U. (1974) Med. Biol. 52: 48—54

Glowinski, J., Iversen, L. L. (1966) J. Neurochem. 13: 658–669

Govoni, S., Missale, C., Battaini, F., Montefusco, O., Trabucchi, M. (1983) Abstr. Joint Meeting Scandinavian Pharmacological Society and Italian Pharmacological Society, p 129

Green, R. D., Proudfit, H. K., Yeung, S. M. H. (1982) Science 218: 58–61

Karasawa, T., Furukawa, K., Yoshida, K., Shimizu, M. (1976) Eur. J. Pharmacol. 37: 97–104

Klawans, H. L., Moses, III, H., Beaulieu, D. (1974) Life Sciences 14: 1493–1500

Lowry, O. H., Rosenbrough, N. J., Farr, A. L., Randall, R. J. (1951) J. Biol. Chem. 193: 265–275

Lloyd, H. G. E., Stone, W. (1981) Pharmacol. Biochem. Behaviour 14: 822–830

Murphy, K. M. M., Snyder, S. H. (1982) Mol. Pharmacol. 22: 250–257

Schwabe, U., Trost, T. (1980) Naunyn Schmiedeberg's Arch. Pharmacol. 313: 179–187

Snyder, S. H., Bruns, R. F., Daly, J. W., Innis, R. B. (1981a) Fed. Prod. Fed. Am. Soc. Exp. Biol. 40: 142–146

Snyder, S. H., Katims, J. J., Annau, Z., Bruns, R. F., Daly, J. W. (1981b) Proc. Natl. Acad. Sci. (USA) 78: 3260–3264

Waldeck, B. (1971) J. Pharm. Pharmacol. 23: 824–830

Ward, H. W., Pollare, T., Geyer, M. (1981) Pharmacol. Biochem. Behaviour 15: 875–877

Williams, M., Risley, E. (1980) Proc. Natl. Acad. Sci. (USA) 77: 6892–6896