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Behavioural and biochemical interaction between caffeine and L-dopa

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The modification by caffeine of the effects of different doses of L-dopa on locomotor activity and catecholamine levels in brain was investigated in mice. Caffeine, which in the dose used caused moderate locomotor stimulation by itself, did not alter the depressant effect on motility of medium doses of L-dopa. The hyperkinetic effect of higher doses of L-dopa was, however, potentiated by caffeine. Biochemically the increase in dopamine levels seen after a dose of L-dopa was enhanced by caffeine. It is suggested that at least part of the potentiating effect of caffeine on the L-dopa induced hypermotility is related to the increased cerebral levels of dopamine.

Recently it has been shown that caffeine increases the yield of [^{3}H]noradrenaline and [^{3}H]dopamine formed from [^{3}H]-L-dopa in the brain of the mouse by a mechanism that did not seem to involve inhibition of the catabolic enzymes monoamine oxidase and catechol-*O*-methyl transferase (Waldeck, 1972). Since caffeine is probably ingested together with L-dopa by many Parkinsonian patients we have looked to see if this biochemical interaction has any functional significance.

We now show that the increased accumulation of dopamine from L-dopa after caffeine is accompanied by functional interaction between the two agents.

MATERIAL AND METHODS

White, female mice, about 24 g, were divided randomly into groups of three. Caffeine (Merck) was dissolved in saline to make 2·5 mg ml^{-1}. L-Dopa (Ajinomoto, Tokyo) was dissolved in a few drops of N hydrochloric acid, diluted with water and the pH of the solution was then adjusted to about 5 with sodium bicarbonate. The final concentration was chosen so that the injected volume was always 40 ml kg.$^{-1}$ All injections were made intraperitoneally. In the behavioural, but not in the biochemical, experiments placebo injections with saline were made so that the injection time-schedule was the same for all groups within an experimental block.

Motor activity was measured by means of a "M/P 40Fc Electronic Motility Meter" (Motron Products, Stockholm, Sweden). This consisted of a horizontal frame holding 40 photoconductive sensors, arranged in 5 rows of 8 cells with a centre to centre distance of 40 mm. The frame was enclosed by a translucent plastic box upon which a Perspex cage was placed. The cage was surrounded by high plywood walls. A 150W incandescent lamp was mounted 115 cm over the photoconductive cells.

Two instruments, placed at a distance of about 50 cm from each other, were used in parallel. Each instrument was connected to an electro-mechanical counter- and timer-unit with automatic print-out of counts. These devices were placed between the two measurement equipments thus allowing the noise from counters and printers to reach the animals in both cages. The counter was set up for a scale of ten and the timer for 5 min periods. When not otherwise stated groups run in parallel received the same treatment.

In the biochemical experiments the animals were killed by decapitation, their brains removed and extracted in 0·4 N perchloric acid. The extracts were put on a Dowex 50 X4 column in the sodium form at pH 6·5. Noradrenaline was then eluted and determined fluorimetrically (Bertler, Carlsson & Rosengren, 1958). Dopamine was recovered from a subsequent fraction and determined fluorimetrically according to Carlsson & Waldeck (1958) as modified by Carlsson & Lindqvist (1962).

In some experiments the effluent from the first column step was acidified to pH 2 and put on another Dowex 50 X4 column, now in the hydrogen form. Dopa was eluted by N hydrochloric acid (Persson & Waldeck, 1970) and estimated fluorimetrically, using the procedure for noradrenaline just described. The recovery of dopa throughout the procedure was relatively low (about 30%). The data have been corrected accordingly.

RESULTS

Behavioural findings

Mice were given caffeine, 25 mg kg^{-1}, 30 min before the administration of L-dopa, 500 mg kg^{-1}. Others received L-dopa only. Immediately after the injection of L-dopa the animals were put into the motility meter cage. In some experiments a group treated with caffeine and L-dopa was run in parallel with a group treated with L-dopa alone. In other experiments parallel groups received either treatment. Animals given caffeine 30 min before measurement were run in parallel with saline controls.

Mice treated with caffeine and L-dopa, gradually developed a hyperactivity reaching a maximum about 25 min after the injection of L-dopa (Fig. 1a and b). During the next 40 min the motility decreased to the same level as after caffeine alone (*cf* Fig. 1c). L-Dopa alone depressed motor activity (Fig. 1b). However, when run in parallel with caffeine pretreated animals, displaying hyperkinesia which increased the noise level from the mechanical counter, animals receiving L-dopa alone showed an increased motor activity following the initial depression (Fig. 1a). This hypermotility reached a peak of about half that of the parallel group and occurred about 15 min later.

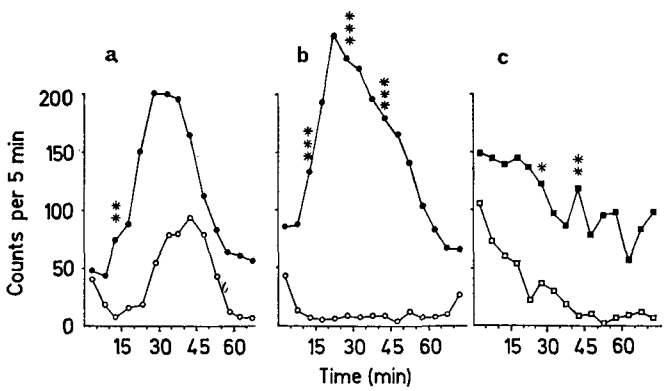

FIG. 1. Effect of caffeine and L-dopa on the motor activity of mice. Caffeine, 25 mg kg^{-1}, i.p., was given 30 min before L-dopa, 500 mg kg^{-1}, i.p., after which the animals were put into a motility meter 3 by 3. Other groups of animals received either drug alone. Controls received saline injections. Motility was measured for consecutive 5 min periods. ● caffeine + L-dopa. ○ L-dopa. ■ Caffeine. □ Control. a and c: Animals with different treatment run in parallel. b: Animals with equal treatment run in parallel. For further explanation, see text. Each point is the mean counts of 4 groups. Significances were tested at 15, 30 and 45 min intervals using Student's *t*-test. Significantly different from caffeine treated animals: * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$.

Normal mice placed in the motility meter cage initially showed a phase of exploratory activity which gradually decreased. After 45 min most movements had ceased. Treatment with caffeine increased the motility. This increase was most striking at the later time intervals when the exploratory activity of the normal mice had ceased. When given alone, caffeine elicited a peculiar kind of stereotyped behaviour, perhaps best described as compulsive grooming. The grooming, which appeared about 1 h after the caffeine injection and lasted for an hour or more, was intense and mostly directed towards another individual. If the object of the activity tried to escape it was dogged by the other. Sometimes one animal, when engaged in grooming another, was in turn subject to the same treatment by the third member of the group. This peculiar behaviour appeared to be masked by the dopa-syndrome in animals which received L-dopa in addition to caffeine.

In the next series of experiments, L-dopa was given in various doses alone or 30 min after the administration of caffeine (25 mg kg^{-1}). After another 20 min the animals were put into the test cage and motility was recorded for 15 min, starting 5 min after the introduction of the animals into the cage. The result is presented in Fig. 2.

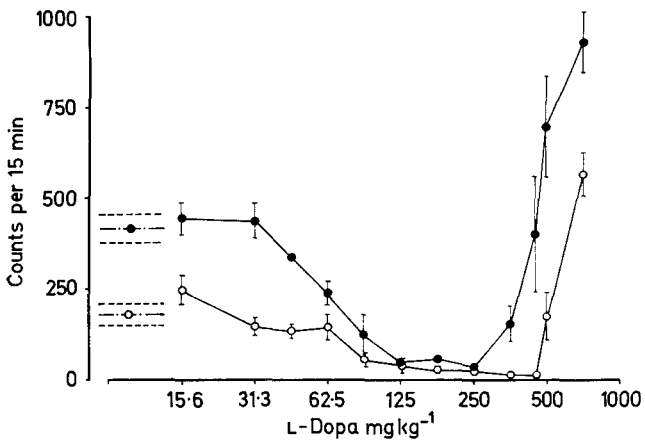

FIG. 2. Effect of caffeine on L-dopa-induced changes in the motor activity of mice. Various doses of L-dopa were given i.p. to mice. Caffeine, 25 mg kg^{-1}, i.p., or saline was given 30 min beforehand. The animals were put into a motility meter, 3 by 3, 20 min after the injection of L-dopa and motility counts were recorded from 5 to 20 min thereafter. ● Caffeine + L-dopa. ○ L-dopa. Shown are the means ± s.e. of 4 groups. The values recorded to the left were obtained in the absence of L-dopa treatment.

Caffeine, when given alone, caused a twofold increase in motility compared with control animals. L-Dopa alone, in doses less than 500 mg kg^{-1}, caused a dose-dependent reduction in motor activity whereas higher doses caused a dose-dependent increase in motility. After caffeine pretreatment, a similar dose-response curve to L-dopa was obtained. However, in this case the shift from depression to hypermotility occurred at a lower dose of L-dopa.

Biochemical findings

Caffeine, 25 mg kg^{-1}, was given intraperitoneally to mice. Thirty min later L-dopa 500 or 125 mg kg^{-1}, was given by the same route and the animals were killed at various time intervals thereafter. Mice, receiving L-dopa alone were run in parallel. Other animals were killed 30 min after caffeine and untreated animals served as controls.

Noradrenaline, dopamine and dopa in the brain were estimated; the result is presented in Fig. 3.

After L-dopa, 500 mg kg^{-1}, dopamine in the brain increased severalfold and appeared to reach a maximum after about 15 min. During the next 45 min it decreased some 30% ($P < 0.005$). In animals pretreated with caffeine dopamine increased further and at the maximum, 30 min after L-dopa, there was about 45% more dopamine than in the animals who received L-dopa alone ($P < 0.01$). Caffeine alone, 30 min after its administration, had no significant effect on the dopamine level in the brain.

There were only slight variations in the level of brain noradrenaline and one way analysis of the variance showed no significant changes. However, a regression analysis of the values obtained 15, 30 and 45 min after the injection of L-dopa revealed that noradrenaline decreased slightly after L-dopa but remained almost constant when caffeine was given in addition, the difference in slope being statistically significant ($P < 0.025$).

Fifteen min after the administration of 500 mg kg^{-1} L-dopa about 20 $\mu g\ g^{-1}$ was recovered from the brain. It seemed to disappear slowly. Caffeine increased the amount of L-dopa recovered. From 15 to 45 min after the administration of L-dopa, the levels of this amino-acid were in general 80% higher in animals pretreated with caffeine ($P < 0.05$, Wilcoxon test).

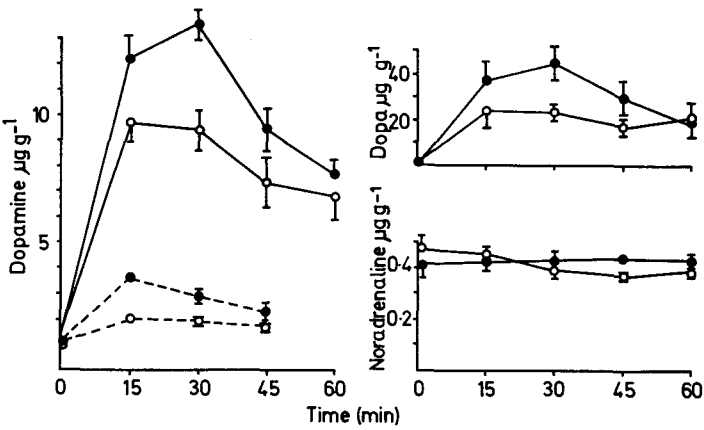

FIG. 3. Effects of caffeine and L-dopa on the levels of dopamine, noradrenaline and dopa in the mouse brain. Mice received L-dopa 500 mg kg^{-1} (solid line) or 125 mg kg^{-1} (broken line), i.p. and were killed at various time intervals thereafter. Half of the animals received caffeine, 25 mg kg^{-1}, i.p., 30 min before L-dopa. Zero time denotes no L-dopa. Shown are the mean ± s.e. of in general 3–4 experimental groups, each comprising 3 animals. ● Caffeine + L-dopa. ○ L-dopa.

After 125 mg kg^{-1} of L-dopa, dopamine in the brain doubled in 15 min ($P < 0.001$). This increase was augmented by caffeine at all time intervals studied ($P < 0.001$, $P < 0.005$ and $P = 0.025$ for 15, 30 and 45 min respectively).

The noradrenaline values obtained after the low dose of L-dopa showed a relatively large scatter and no significant changes in mean values. These data are not shown.

DISCUSSION

In accordance with previous studies, caffeine, 25 mg kg^{-1}, when given alone increased the motor activity (Boissier & Simon, 1965; Hertz, Neteler & Teschemacher, 1968; Thithapandha, Maling & Gillette, 1972). This stimulation was reversed into a marked depression by L-dopa in doses about 200 mg kg^{-1}, *i.e.* the depression caused by L-dopa given in this dose was not counteracted by caffeine. The interpretation of this reversal must wait until the nature of the depressive phase of L-dopa action and the mechanism of the caffeine-induced stimulation have been elucidated.

When the dose of L-dopa was increased, the depression changed into hypermotility (*cf.* Boissier & Simon, 1966 and Strömberg, 1970.) This shift occurred at lower doses in animals pretreated with caffeine than in those that received the amino-acid alone. In doses near those inducing reversal, the animals appeared to be hypersensitive to external stimuli. Thus, when a group treated with L-dopa in a depressive dose was run in parallel with a group which received caffeine in addition, the former group also showed an increased motility but with a lower maximum which occurred about 15 min later (Fig. 1). This phenomenon is probably due to acoustic stimulation from the mechanical counter (see materials and methods) since it was not observed when parallel groups received L-dopa alone: when the caffeine pretreated group started to activate the counter the parallel group was acoustically stimulated and started to move. It is known that L-dopa increases the susceptibility to external stimuli (Carlsson, 1972).

This hyperexcitability and the different experimental conditions may also explain why animals receiving 500 mg kg^{-1} of L-dopa did not show the same degree of motility depression in the dose-curve experiments (Fig. 2) as in the time-curve experiments (Fig. 1b). In the latter case the mice were moved into the test cage immediately after the L-dopa injection whereas in the former case the animals were moved 20 min after the injection *i.e.* at a time when they were influenced by L-dopa.

After L-dopa, 500 mg kg^{-1}, dopamine in the brain increased severalfold, and after pretreatment with caffeine there was a further increase in brain dopamine. The appearance of this "additional" amount was contemporaneous with the hypermotility elicited by caffeine and L-dopa (*cf.* Fig. 1 with Fig. 3).

The question now arises as to whether there is a causal relation between the appearance of excessive brain dopamine and the temporally related hypermotility. L-Dopa given systematically is partly lost during the passage through the brain capillaries where it is decarboxylated to dopamine, which in turn is destroyed by the monoamine oxidase (Bertler, Falck & Rosengren, 1963). Recently, serious doubt has been raised as to whether L-dopa is at all able to penetrate this enzymatic barrier (de la Torre, 1972). Consequently, the excessive dopamine found biochemically in the brain after an acute dose of L-dopa should be located in the capillary lumen and in the endothelial cells, and thus unable to reach the neuronal tissue (de la Torre, Boggan & Lovell, 1972). This suggestion was based mainly on qualitative histochemical observations. However, it has been demonstrated by both histochemical and biochemical means that brain catecholamine stores depleted by inhibition of the tyrosine hydroxylase, are promptly refilled by an injection of L-dopa. On the other hand, when depletion was caused by reserpine, which blocks the amine-storage mechanism of the adrenergic neurons, no refillment was obtained (Corrodi, Fuxe & Hökfelt, 1966).

Further, there are numerous indications that a significant part of the dopamine found biochemically in the brain after an acute dose of L-dopa reaches neuronal

tissues. Firstly, the excess dopamine found after a large dose of dopa is concentrated in the caudate nucleus while the cerebellum shows a low concentration (Bertler & Rosengren, 1959). This is in spite of an almost uniform distribution of the fluorescent capillaries (Bertler & others, 1963). Moreover, labelled L-dopa given in tracer amounts is recovered from the caudate nucleus as labelled dopamine in concentrations ten times higher than from the hemispheres or from the spinal cord (Persson, 1969). Secondly, 2 h after the administration of 100 mg kg^{-1} L-dopa about 30% of the brain noradrenaline is derived from the injected precursor, as elucidated with isotope techniques (Persson & Waldeck, 1971). Since the total noradrenaline level was almost unchanged, this indicates that dopamine derived from injected L-dopa reaches the noradrenaline-containing neurons where it is hydroxylated to noradrenaline and displaces the endogenous stores. Finally, the half lives of labelled catecholamines formed from labelled L-dopa in the brain are equal to those of catecholamines formed from labelled tyrosine, the natural precursor (Persson, 1969).

Thus it is established beyond doubt that after an acute dose of L-dopa the amino-acid is converted to catecholamines which can be taken up and stored by the noradrenaline- and dopamine-containing neurons in the brain. It remains to be elucidated, however, to what extent L-dopa penetrates the enzymatic blood-brain barrier unchanged, for subsequent decarboxylation by neuronal decarboxylase; and how much of the dopamine formed in the capillary walls will diffuse into the parenchyma. A recent investigation (Andén, Engel & Rubenson, 1972) shows that both alternatives are possible.

Consequently, it is reasonable to assume that at least part of the potentiating effect of caffeine on the L-dopa-induced hypermotility is related to increased cerebral levels of dopamine. The increased dopa levels suggest a facilitation of the uptake of L-dopa. However, other mechanisms for the potentiating effect of caffeine are possible, e.g. receptor sensitization. Recent investigations indicate that caffeine interacts with central catecholamines in a complex manner (Berkowitz, Tarver & Spector, 1970; Waldeck, 1971; Corrodi, Fuxe & Jonsson, 1972).

Caffeine increased the yield of dopamine in the brain also after 125 mg kg^{-1} L-dopa, although the animals were still depressed. Previous studies suggest that brain dopamine must increase above a certain threshold level before the depression produced by L-dopa administration changes to hyperactivity (Butcher, Engel & Fuxe, 1972). Caffeine appears to reduce the dose of L-dopa required to reach this critical level.

Brain noradrenaline, if anything, decreased after L-dopa, 500 mg kg^{-1}, but remained constant when caffeine was given in addition. When large doses of L-dopa are given, dopamine β-hydroxylase appears to become saturated (Persson & Waldeck, 1971). Therefore, the production of noradrenaline cannot cope with the releasing action of the excessive amounts of dopamine. There is a possibility that caffeine in some way facilitates either the production or storage of noradrenaline, or both.

L-Dopa in doses near the point at which saturation of dopamine β-hydroxylase appears to occur may give either increased, unchanged or reduced brain levels of noradrenaline. This will explain some contradictory results found in the literature concerning the effect of L-dopa on brain noradrenaline, as well as the somewhat erratic results when 125 mg kg^{-1} L-dopa was given in the present investigation.

The main conclusion that can be drawn from the present results is that caffeine interacts with L-dopa, both functionally and biochemically. The possibility should

thus be considered that caffeine ingested, e.g. in beverages, may alter the therapeutic response to L-dopa.

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