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The effect of caffeine on the mitosis of human lymphocytes in culture

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Summary

1. The effect of caffeine at concentrations of 10^{-2}M to 10^{-4}M on the mitotic rate of phytohaemagglutinin-stimulated human lymphocytes from twelve normal subjects was examined *in vitro*.

2. At 10^{-2}M the drug was cytotoxic.

3. At 10^{-3}M caffeine reduced the rate of mitosis in all cases.

4. At 10^{-4}M the drug was found to reduce the rate of mitosis in some cases, to give the same value as the control cultures in others and in a third group to increase the mitotic rate.

5. At 10^{-4}M all the lymphocytes from females showed an increased rate of mitosis and all but one of the lymphocyte samples from males showed a decrease or no change. Thus at this concentration the effect of the drug on the mitotic rate appeared to be related to the sex of the lymphocyte donor. There was also a significant difference in the degree of inhibition between male and female lymphocytes at 10^{-3}M.

Introduction

Caffeine is known to be mutagenic in at least some micro-organisms, for example in the fungus *Ophiostoma* (Fries & Kihlman, 1948 ; Fries, 1950 ; Zetterberg, 1960), and in the bacterium *Escherichia coli* (Demerec, Bertani & Flint, 1951 ; Gezelius & Fries, 1952). It is known to be lethal to plant cells at high concentrations (1% and 2%) and to suppress the mitotic rate at lower concentrations (Kihlman & Levan, 1949). There has been much speculation on the possible mutagenic effect of caffeine in man because it has been shown to reach the gonads and most of the population have a small daily intake of the drug (Schull, 1962). Caffeine-withdrawal can cause headache in susceptible individuals and it is a component of some headache remedies (Dreisbach & Pfeiffer, 1943). It is actively metabolized in man since only 0·5 to 1·5% of administered caffeine is excreted unchanged and it is distributed throughout the body tissues in approximate proportion to their water content (Axelrod & Reichenthal, 1953). The same authors estimated the average half-life of caffeine in man at 3·5 h. After caffeine administration to human volunteers approximately equal amounts of methylxanthines and methyluric acids are present in the urine (Cornish & Christman, 1957).

With normal intakes, therefore, there will be no day to day accumulation of caffeine in the body but it is possible that large doses might affect the mitotic rate of human cells. This possibility has been investigated by studying the action of caffeine on phytohaemagglutinin (PHA)-stimulated human lymphocytes *in vitro*.

Methods

Venous blood samples were obtained from healthy adults. No attempt was made to get them to abstain from caffeine-containing drinks before the samples were taken, since it was intended to study the effect on lymphocytes exposed to the usual caffeine intake of the population sample. The blood was immediately placed in a sterile bottle containing anticoagulant (heparin in dextran) and after allowing the erythrocytes to settle the supernatant plasma and cells were withdrawn. 1·5 ml of the cell-plasma suspension was made up to 10 ml with TC 199 (Glaxo) for each culture. TC 199 is a tissue culture medium containing the basic materials for cell growth (Morgan, Morton & Parker, 1950). It contains no caffeine. Caffeine (Sigma, purity at least equivalent to USP) dissolved in TC 199 was added to the experimental cultures to give final concentrations of 10^{-2}, 10^{-3}, and 10^{-4}M. Control cultures with no added caffeine were set up at the same time. Two drops of reconstituted PHA P (Difco) were added at the start of the culture period and after 72 h incubation at 37° C, 0·2 ml of a solution of 1 mg of demecolcine (" Colcemid ", Ciba) in 100 ml of TC 199 was added. After 2 h at 37° C, the cells were spun down, resuspended in hypotonic saline for 15 min at 37° C, spun down and fixed in two changes of acetic acid-alcohol. They were then resuspended in 45% acetic acid, spread on to cold slides, air-dried and stained with 10% Giemsa at pH 6·4. One thousand cells from each culture were examined and the number of mitoses recorded. These results were then expressed as a percentage of the control value. The average control value was 17 mitoses per 1,000 cells.

Results

Lymphocytes from twelve individuals were cultured as described. At 10^{-2}M caffeine in all cases no transformation of the lymphocytes in response to PHA-stimulation was observed and no mitoses were seen, which indicates that caffeine is cytotoxic at this concentration. At 10^{-3}M caffeine there was inhibition of mitosis

TABLE 1. *Effect of caffeine on the mitosis of phytohaemagglutinin-stimulated human lymphocytes*

Response to 10^{-4}M caffeine	Inhibition	Stimulation	Unchanged	Total
Number of individuals	4	6	2	12
Conc. of caffeine	Mitosis as % control in the above individuals			Mean
10^{-4}M	32	200	100	111
10^{-3}M	18	55	74	41
10^{-2}M	None	Cells killed		

Results are classified by the action of the drug at 10^{-4}M on the rate of mitosis.

TABLE 2. *Effect of caffeine on the mitosis of phytohaemagglutinin-stimulated human lymphocytes*

Sex of lymphocyte donor	Male	Female	Total
Number	7	5	12
Conc. of caffeine	Mitosis as % control		Mean
10^{-4}M	68	192	111
10^{-3}M	28	65	41
10^{-2}M	None	Cells killed	

Results are classified by the sex of the lymphocyte donor.

in all cases, while at 10^{-4}M in some cultures inhibition was observed, in others the control value was obtained and in a third group stimulation of the mitotic rate occurred. The averaged results, classified by the action of caffeine at 10^{-4}M on the mitotic rate, are given in Table 1. A regrouping of the data by the sex of the lymphocyte donor revealed that all the females were in the stimulated group and all but one of the males were in the inhibited or unchanged groups. The results classified by the sex of the lymphocyte donor are given in Table 2.

The difference in the effect of caffeine at 10^{-3}M and 10^{-4}M on the mitotic rate of lymphocytes from males and females has been tested statistically by means of the ranking method of Heath & Irwin (1962). Using the null hypothesis that there is no difference due to the sex of the lymphocyte donor this analysis gave the following results: for 10^{-3}M, $P=0{\cdot}021$ and for 10^{-4}M, $P=0{\cdot}0034$, both of which are significant. It is therefore reasonable to reject the null hypothesis and conclude that the effect of caffeine on the mitosis of human lymphocytes in culture is related to the sex of the lymphocyte donor.

Discussion

Caffeine at a final concentration of 10^{-2}M was found to be cytotoxic to the lymphocytes of all the donors. This is equivalent to a dose of 97·1 g of caffeine for a 70 kg man in order to achieve a concentration of 10^{-2}M in his total body water. Since a 125 ml cup of coffee contains 95 to 125 mg of caffeine and a 150 ml cup of tea 60 to 90 mg it is impossible to reach the cytotoxic level by tea or coffee drinking. At 10^{-3}M caffeine acted as a mitotic inhibitor in all cases, although the inhibition was significantly greater in males than in females. To reach this concentration in his total body water a 70 kg man would need a dose of 9·71 g of caffeine, which is approximately equivalent to 80 cups of coffee or 110 cups of tea. Since caffeine is rapidly metabolized in the body with an estimated half-life of only 3·5 h it is unlikely that this concentration would ever be reached by drinking coffee or tea. At 10^{-4}M the effect also appears to be related to the sex of the lymphocyte donor. In order to achieve this concentration in his total body water a 70 kg man would need a dose of 0·971 g of caffeine or to drink eight cups of coffee or eleven cups of tea within a short time. The caffeine content of analgesic preparations is usually between 30 and 50 mg per tablet, and therefore twenty to thirty tablets would need to be taken to equal a dose of 0·971 g. From these considerations it would appear that it is unusual for an antimitotic dose of caffeine to be taken during normal exposure to the drug although some individuals must on occasion be exposed to doses equivalent to 10^{-4}M for a short time.

The difference in the response of male and female lymphocytes to 10^{-3}M and 10^{-4}M caffeine in culture has no immediately obvious explanation. It may be that there is some synergistic effect between the traces of sex hormones in the plasma used and caffeine. It is possible that the caffeine is able to derepress some mitosis-controlling locus on the *X* chromosome and is thus able to have a greater effect on female cells.

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