

THE EFFECT OF HIGH DOSES OF CAFFEINE (30 mg/1000 g BODY WEIGHT) ON BLOOD AMYLASE ACTIVITY OF MALE RATS

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The studies of numerous authors (7, 8) have established that small doses of caffeine (8 mg/1000 g) increase the inhibitory processes, whereas moderate doses (20 mg/1000 g) and large doses (60 mg/1000 g body weight) increase the processes of excitation in the central nervous system.

The parenteral administration of a moderate doses of caffeine (15 mg/1000 g body weight) gives rise to an increase of amylase activity in the blood of male rats (6). We assumed that under the influence of the created process of excitation in the central nervous system the secretion of androgen hormones from the testes and adrenal cortex is increased. Androgen hormones and testosterone in particular as we have already shown (5) activate amylase *in vivo*.

On the other hand, *in vitro* experiments Vincent D. and Lagreu R. (28) have established an inhibitory effect of caffeine in concentrations higher than M/200 on salivary, pancreatic and malz amylase. Concentrations of M/200 and less display no effect. In a previous paper (6) we reported of the administration of 15 mg/1000 g caffeine, which corresponds to a concentration lower than M/200.

In the present work we set ourselves the task to study the effects of higher doses of caffeine (30 mg/1000 g body weight) on amylase activity in the blood in order to establish whether the observed *in vitro* effect would occur *in vivo*.

Data lack in the available literature on studies of the caffeine effect on amylase *in vivo*.

Method

Studies were carried out with two groups of albino male rats weighing from 160 to 210 g; the first group — control animals (15 rats), the second group — subcutaneously injected with caffeine in doses of 30 mg/1000 g body weight (15 rats). The latter were examined 3 hours after the caffeine administration. Control and experimental animals were killed by decapitation in one and the same hour of the day, no food being given 15 hours before decapitation. The method of investigation is described in detail in our previous papers (5, 6).

Amylase activity in the blood was determined by the increase of reducing substances after incubation with starch — after Kalitsin's modification (4) of the method of Engelhardt and Gerchuk (9). Each experi-

ment was performed with two parallel blood samples for incubation purposes. From each work we had 2 complete and two control experiments. The results obtained represented the mean arithmetical value of 4 parallel estimations.

Results

The experimental data thus obtained were calculated after the methods of variational statistics (1, 2). The results are presented on the following table.

Table 1

Amylase activity in the blood of control and injected with caffeine (30 mg/1000 g body weight) male albino rats.

Incubation 2 hours at 37°C, pH — 6.5

Amylase activity is expressed by means of the increase in reducing substances in mg to 1 ml of blood

No of experiment	Increase in reducing substances (mg/1 ml)	
	Controls	Animals injected with caffeine
1	82,4	62,4
2	104,8	102,4
3	96,8	75,5
4	136,8	103,6
5	142,8	69,5
6	75,8	89,0
7	109,6	105,3
8	147,6	72,5
9	82,8	83,0
10	125,8	108,4
11	96,4	89,2
12	95,0	67,6
13	131,6	65,8
14	86,2	72,8
15	126,4	68,3
X	109,3	82,4
±S	21,3	16,3
±SX	5,8	4,2

X — mean arithmetical value resulting from the different experiments.

SX — mean square error of the mean arithmetical valuations.

The data from these studies indicate that amylase activity in the blood of male rats, injected with 30 mg/1000 g body weight caffeine is by 24,7% lower as compared with control male rats.

The established differences are statistically reliable calculated at new ones at a probability equal to 0,05.

Discussion of results

In the present work we find suppression of amylase activity in the blood of male rats injected with 30 mg/1000 g body weight caffeine. At such a dose an excitation is created in the central nervous system (8). For that reason we suppose that suppression of amylase activity in this

case is achieved not through central neuro-humoral routes but rather through a direct action of caffeine.

The probability of such a direct action *in vivo* is supported by the results of Vincent et Lagreu (28). The administration of higher doses of caffeine lead us closer to the limiting concentration of M/200 beyond which the abovementioned authors establish an inhibitory effect upon amylase activity (lower concentrations exhibit no effect). We may assume that with higher concentrations of caffeine a direct inhibitory effect predominates over the activating effect exerted via indirect routes and effectuated through the central nervous system and androgen hormones (6).

The mechanism of the inhibitory effect of caffeine on amylase activity is not elucidated so far (28). Blood amylase activity exhibits a close relation to amylase in the cells of the pancreas where its biosynthesis takes place. It is even supposed that blood amylase is identical with pancreatic amylase and originates mainly from the pancreas (19). On the basis of such assumptions we might suggest that one of the reasons for the decrease in blood amylase activity lies in a disorder of amylase biosynthesis in the pancreas under the influence of higher doses of caffeine.

It is likely that caffeine — 1, 3, 7-trimethylxanthin, with a structure close to that of purines, introduced in larger amounts may exert the effect of an analogue-antagonist and so lead to the suppression of protein synthesis.

Our suggestions are based on the results from Druckrey's and Schreiber's studies (15). These authors have established that the growth of tissue cultures is inhibited by high doses of caffeine. Stieve and Eichler (27) have observed growth inhibition in rabbits and rats, treated with high doses of caffeine — above 100 mg/1000 g body weight. Holek thinks that this dose is toxic (18). The above mentioned authors (15, 27) give no explanation of this inhibiting effect.

On the other hand direct evidence exists at the present which witnesses the participation of nucleic acids in protein synthesis (25, 26). Attempts to disturb nucleic acid synthesis by means of purine and pyrimidine analogues lead to the inhibition of protein synthesis (3, 13, 26). The following analogues of the purines exhibit inhibitory effect: 2, 6 diaminopurine (17, 24), 8-azaguanine (13, 16, 20), 6-merkaptopurine (20, 23). An inhibitory effect is also observed of hypoxantin on protein synthesis (21).

It is established that the caffeine is demethylated in the organism: in the human — to theophyllin, in the dog — to 3 methylxanthin (10). It may be assumed that under certain conditions caffeine or more probably some of its metabolites may appear to be an antagonist of purine and of protein synthesis, resp. thus exerting an inhibitory effect upon the enzymes.

Data of the present work are only preliminary. Further studies are necessary as regards other enzymes as well as in other conditions with more direct investigations on protein synthesis, in order to study the effect of caffeine as analogue of purines.

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ВЛИЯНИЕ БОЛЬШИХ ДОЗ КОФЕИНА (30 МГ/КГ) НА АКТИВНОСТЬ АМИЛАЗЫ В КРОВИ КРЫС МУЖСКОГО ПОЛА

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РЕЗЮМЕ

Было исследовано *in vivo* влияние больших доз кофеина (30 мг/кг веса) на активность амилазы в крови крыс мужского пола. Опыты проводились на 14 контрольных и 10 третируемых кофеином крысах.

Анализ активности определялся по разработанной Калициной (4) модификации метода Энгельгардта и Герчука (9).

Данные исследований, подвергнутые статистической обработке, показывают, что под действием кофеина в дозе (30 мг/ кг веса), активность амилазы в крови понижается на 24,7 процентов в отношении активности у контрольных крыс.

Для объяснения ингибирующего эффекта кофеина на амилазу *in vivo*, допускается возможность антагонистического действия кофеина или каких нибудь его метаболитов в отношении белкового, соответственно энзимного биосинтеза.