DOSE- AND TIME-DEPENDENT LIPOLYTIC EFFECT OF AMPHETAMINE IN EXPERIMENTAL ANIMALS

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Amphetamine effect on lipid metabolism is an object of numerous investigations conceived mainly by its manifested anorexigenic influence and stimulating action on mental and physical working capacity. Amphetamine increases free fatty acids (FFA) in plasma which is an evidence for the influence of this drug on lipolytic processes (5, 7, 8, 10). It is suggested that amphetamine lipolytic effects are related to noradrenalin liberation from adrenergic neurons, to an increased adrenalin secretion from the adrenal medulla or that they are of central origin (9).

There are scanty investigations of details of amphetamine lipolytic action. According to Bizzi et al. (5) amphetamine does not induce FFA concentration changes in the adipose tissue. No data about its action on single lipid classes (triglycerides, cholesterol esters, phospholipids) as well as about its influence on individual plasma FFA liberation are available. Our earlier studies in man showed an outlined selectivity of amphetamine lipolytic action related to arachidonic acid liberation (2).

The present investigation is directed towards further characterization of amphetamine lipolytic effect. Dose- and time-dependence of this effect is examined concerning the main plasma FFA: $C_{14:0}$ (myristic acid), $C_{16:0}$ (palmitic acid), $C_{16:1}$ (palmitoleic acid), $C_{18:0}$ (stearic acid), $C_{18:1}$ (oleic acid), $C_{18:2}$ (linolic acid), $C_{20:3}$ (eicosotrienic acid) and $C_{20:4}$ (arachidonic acid).

Material and methods

The study covered 94 white male rats with 150—170 g b. w. Amphetamine was applied at doses of 5, 10 and 20 mg/kg b. w. intraperitoneally. Effect was controlled on the 30th min with all the doses used but also on the 15th and 60th min after drug administration with the dose of 10 mg/kg b. w. Blood for examination was taken by means of sublingual vein incision. Control animals given saline injections were parallelly killed with corresponding doses and time-intervals of experimental ones.

Blood plasma was extracted according to the method of Folsh et al. (6). Lipid extract obtained was processed by using of thin-layer chromatography in the system hexane:ether:acetic acid (3). FFA were methylized by diazomethan synthesized in our laboratory. Methyl esters of the fatty acids were analysed by means of gas-liquid chromatography (gas chromatograph «Chrom 4»).

Results and discussion

Our data demonstrate that amphetamine lipolytic action is a dose-dependent phenomenon. Total FFA concentration increased by 19 per cent, 56 per

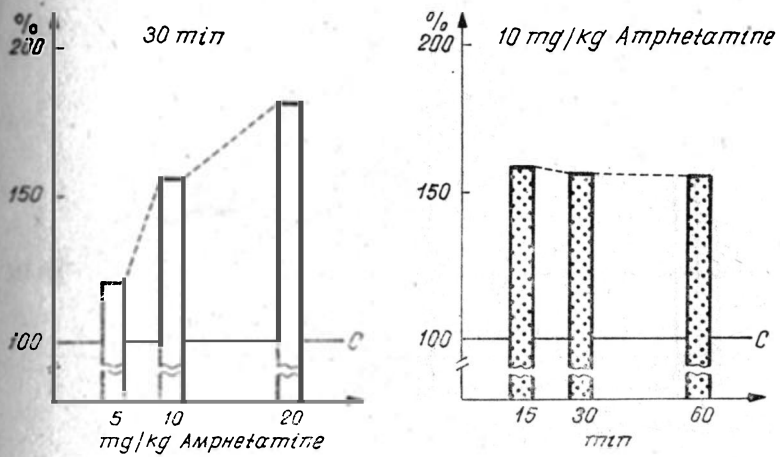

Fig. 1. Plasma FFA increase after amphetamine treatment (control values are 100 per cent)

cent ($p<0.05$), and 78 per cent ($p<0.01$) with the corresponding doses used (5, 10, and 20 mg/kg b. w.) (fig. 1). The effect was manifested already on the 15thmin and then remained constant during the next two intervals (fig. 1). FFA increase was only by 10 per cent on the 120th min. This fact restricted our examination up to the 60th min after amphetamine application only.

Amphetamine liberated predominantly unsaturated fatty acids in the three different doses used: $C_{16:1}$, $C_{18:1}$, $C_{20:3}$, $C_{18:2}$, $C_{20:4}$. The selective lipolysis was most pronounced when the dose of 10 mg/kg b. w. 30 min after drug introduction was concerned (fig. 2). The character of lipolytic effect remained constant with 15 min interval, however, to a lower extent whereas saturated/unsaturated acid ratio changes in favour of the saturated ones with 60 min interval (fig. 3). This change gave reason to suggest that substrates rich in saturated fatty acids were involved in the lipolytic process when interval of 60 min was concerned.

FFA concerntrations in amphetamine doses used increased to a different degree. Changes were most considerable in the dose of 10 mg/kg b. w. The following FFA were most sharply elevated: $C_{20:3}$ — by 121 per cent; $C_{18:1}$ — by 95.6 per cent; $C_{20:4}$ — by 78 per cent, and $C_{18:2}$ — by 52.4 per cent. This relatively selective lipolysis could be due to the activation of phospholipases A_2/A_1 attacking membrane phospholipids rich in these fatty acids. Changes of single FFA were within narrower limits when dose of 20 mg/kg b. w. was applied: from 65 per cent (for $C_{18:0}$) up to 95.8 per cent (for $C_{16:1}$). It seemed possible that lipolytic enzymes liberating fatty acids from α-position of the triglycerides were also activated when this high dose was administered (fig. 4).

Time-effect dependence shows different tendencies with single fatty acids. Both $C_{20:4}$ and $C_{20:3}$ curves had similar pattern with a peak on the 30th min and pronounced fall on the 60th min. In comparison with their peak levels concentrations. The FFA $C_{20:3}$ and $C_{20:4}$ decreased by 120.6 per cent and 64.8 per cent, res-

pectively, on the 60th min. Rapid consumption of these fatty acids from FFA pool reflects probably the accelerated metabolism of these polyunsaturated fatty acids in direction towards oxidation, synthesis of prostaglandins and other biologically

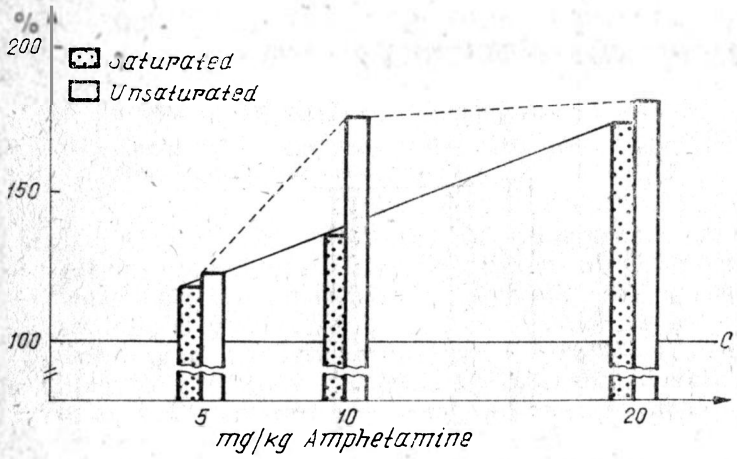

Fig. 2. Saturated and unsaturated fatty acid increase depending on applied dosis of amphetamine (control values are 100 per cent)

active substances as well as towards phospholipid and triglyceride resynthesis (1). This presumption was confirmed by previously reported extreme arachidonic

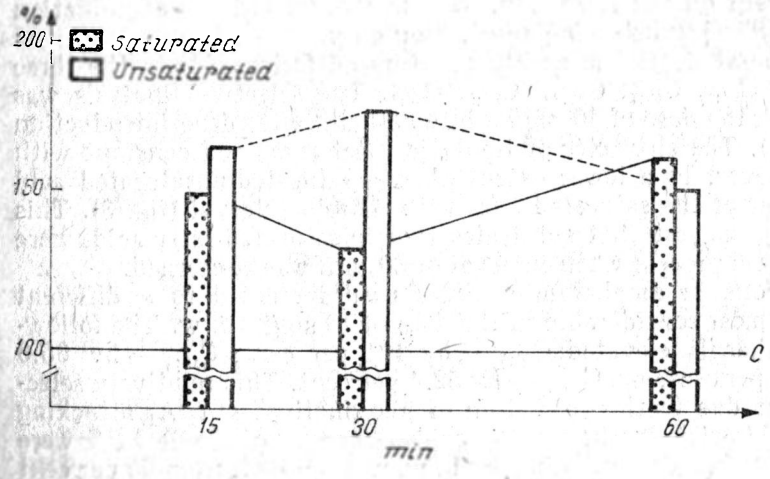

Fig. 3. Saturated and unsaturated fatty acid increase after 10 mg/kg amphetamine (control values are 100 per cent)

acid increase in man under the influence of amphetamine on the background of acetysal-inhibited cycloxygenase (2). During this blockade lipoxygenase remained active and caused endoperoxides' accumulation which stimulated on its part lipolysis and ensured arachidonic acid increase (4). Major fatty acid increase,

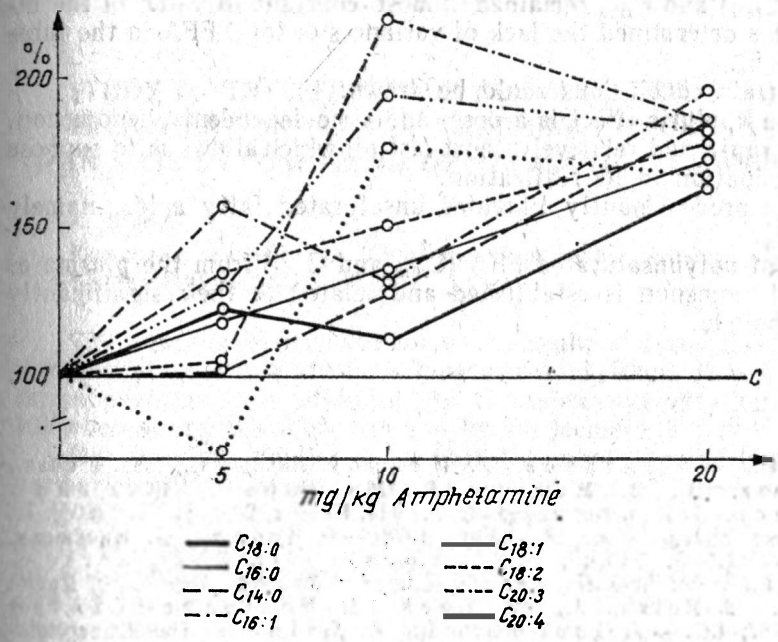

Fig. 4. Plasma FFA increase 30 min after 5, 10 and 20 mg/kg amphetamine (control values are 100 per cent)

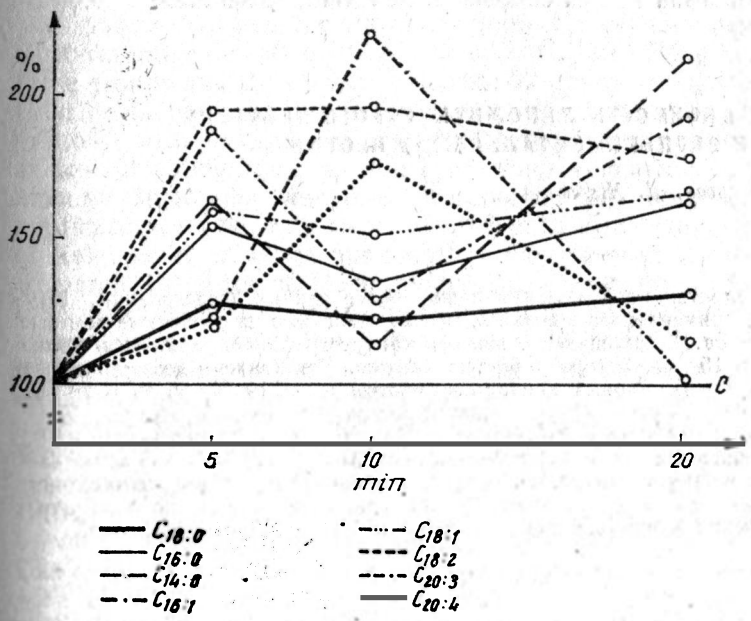

Fig. 5. Plasma FFA increase after 10 mg/kg amphetamine (control values are 100 per cent)

namely $C_{16:0}$, $C_{18:2}$, $C_{18:1}$, and $C_{18:0}$ remained almost constant in value in the intervals studied. This determined the lack of variances of total FFA in the three time intervals (fig. 5).

The following main conclusions could be drawn:

1. Amphetamine lipolytic effect is a dose- and time-dependent phenomenon.
2. The effect is rapid and relatively short-lasting which allows us to suppose catecholamine participation in its realization.
3. Amphetamine predominantly liberates unsaturated fatty acids, namely $C_{20:3}$, $C_{20:4}$, and $C_{18:2}$.
4. A depletion of polyunsaturated FFA ($C_{20:3}$ and $C_{20:4}$) from the plasma as a time-dependent phenomenon is established and related to their significantly more active metabolism.

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ДОЗА- И ВРЕМЯ-ЗАВИСИМОСТЬ ЛИПОЛИТИЧЕСКОГО ДЕЙСТВИЯ АМФЕТАМИНА ПРИ ЭКСПЕРИМЕНТАЛЬНЫХ ЖИВОТНЫХ

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РЕЗЮМЕ

Настоящая работа является продолжением наших более ранних исследований. Изучено влияние амфетамина, применяемого в дозах 5, 10 и 20 мг/кг веса на свободные жирные кислоты у белых крыс-самцов. С помощью газ-жидкой хроматографии определялось количество 8 жирных кислот в 15-тую, 30-тую и 60-тую минуты. Установлено доза-зависимое увеличение тотальных свободных жирных кислот соответственно на 19 %, 56 % и 78 %. Эффект хорошо выражен еще в 15-ую минуту и остается постоянным до 60-ой минуты. Увеличение индивидуальных жирных кислот неравномерно. При всех дозах и интервалах наиболее высоко увеличена линолевая кислота (до 95 %). Максимальное увеличение арахидоновой кислоты отмечается в 30-ую минуту. Ненасыщенные жирные кислоты значительно меньше увеличены. Полученные данные показывают наличие избирательности в липолитическом действии амфетамина преимущественно к ненасыщенным жирным кислотам.