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SUBSTRATE SUPPLY AND ENERGY METABOLISM OF SKELETAL MUSCLE OF MICE TREATED WITH METHAMPHETAMINE AND PROPRANOLOL

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Abstract—In order to examine, whether the inhibition by propranolol of methamphetamine-induced motor excitation has to be ascribed to lack of substrate supply and inadequate energy production in skeletal muscle, the influence of 3 μg/g methamphetamine subcutaneously (s.c.) alone or in combination with 5 μg/g propranolol intraperitonially (i.p.) on liver glycogen, blood glucose, non-esterified fatty acids in the blood and the glycogen, pyruvate, lactate, ATP and phosphocreatine contents of skeletal muscle has been investigated in mice. The following results have been obtained: (1) Methamphetamine stimulates lipolysis in adipose tissue and glycogenolysis in liver and muscle and thus provides the substrate required for an adequate energy production in skeletal muscle. (2) Propranolol inhibits methamphetamine-stimulated lipolysis and at the same time enhances the mobilization of hepatic glycogen and the utilization of carbohydrates in skeletal muscle. In this way enough substrate can be supplied to skeletal muscle to maintain constant ATP and phosphocreatine levels. It is concluded from the results, that the inhibition by propranolol of methamphetamine-induced excitation is not due to an inhibition of energy metabolism of skeletal muscle.

IN AN earlier paper from this laboratory[1] we have shown, that in mice propranolol significantly reduces the motor excitation produced by administration of methamphetamine. Propranolol is known to exert a great number of peripheral and central actions, e.g. sedation, blockade of adrenergic receptors within the central nervous system[2-6] and inhibition of the mobilization of the fat and carbohydrate stores,[7-9] which could be responsible for its antagonism to methamphetamine, but in our previous report it had to remain unsettled, which of them was the real cause of the methamphetamine antagonism. Since under certain conditions, probably by inhibiting lipolysis and glycogenolysis and thus reducing the amount of substrate available for muscular activity, propranolol can greatly reduce the animals' ability for physical work,[10] the experiments presented in this paper were performed in order to examine, whether mice treated with methamphetamine + propranolol would exhibit a lack of energy of such kind, that it might be made responsible for the reduced motor activity. If this eventuality could be excluded, our experiments would provide strong evidence that the methamphetamine antagonistic effect had to be ascribed not to the peripheral but to the central nervous system effects of propranolol. Therefore, in order to get insight into the substrate supply and energy reserves of muscular tissue, liver glycogen, blood glucose, non-esterified fatty acids in the blood (NEFA) and the contents of glycogen, pyruvate, lactate, ATP and phosphocreatine in skeletal muscle of the legs of mice treated with methamphetamine and propranolol were determined.

METHODS

All experiments were performed on NMRI mice of either sex, which were kept at 25° and fed with standard diet (Altromin R) and tap water *ad lib*. The animals were treated with 3 μg/g methamphetamine HCl s.c. or with 5 μg/g *dl*-propranolol HCl i.p. or with both drugs simultaneously. Over a period of 2 hr the motor activity of the animals was recorded in circular activity cages, as described previously.[3] One or 2 hr after the drug administration animals were killed by immersion in liquid air or by decapitation.

Samples of liver and muscular tissue from the hind legs were obtained from the animals frozen *in toto*. They were dissected while the animals were still being frozen. Glycogen was determined according to Kemp and Kits van Heijningen,[11] pyruvate according to Bücher,[12] lactate according to Scholz *et al.*,[13] ATP and phosphocreatine according to Lamprecht and Stein.[14] Blood for the determination of glucose and non-esterified fatty acids was drawn from the decapitated animals. Glucose was estimated according to Huggett and Nixon,[15] non-esterified fatty acids according to Duncombe.[16]

Mean values were calculated from nine to 26 single determinations. The results were checked statistically by means of Student's *t*-test. Differences between two mean values were regarded to be significant if $P \leqq 0{\cdot}05$.

RESULTS

Methamphetamine

Three μg/g methamphetamine s.c. greatly increase the running activity of mice for at least 2 hr (Fig. 1). During this time the glycogen content of the liver shows a

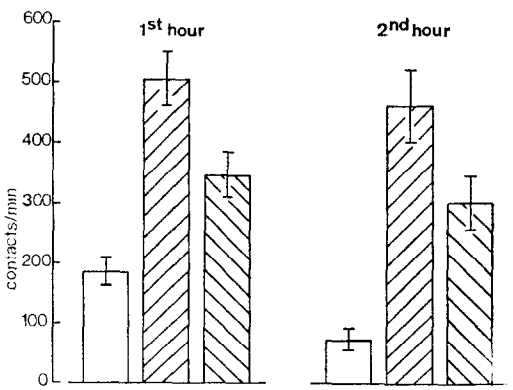

FIG. 1. Spontaneous motor activity of mice. Mean values of 24 single determinations and S.E. □: Controls, ▨ 3 μg/g methamphetamine s.c., ▧ 5 μg/g propranolol i.p. + 3 μg/g methamphetamine s.c.

continuous decline. The blood glucose level is raised with maximum values after 1 hr. The non-esterified fatty acids in the blood show a similar pattern as the blood glucose (Fig. 2). In skeletal muscle the glycogen content is decreased by 33 per cent within 1 hr, but there is no further decrease within the second hour of methamphetamine treatment. The lactate content of the muscular tissue shows a decrease only in the second hour.

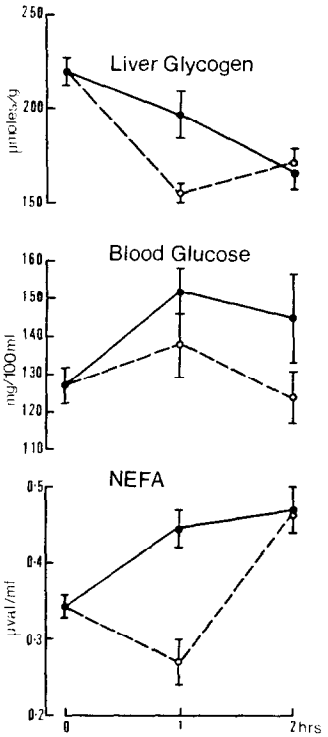

FIG. 2. Liver glycogen (given as μmoles glucose equivalents/g), blood glucose and non-esterified fatty acids in the blood (NEFA) of mice treated with 3 μg/g methamphetamine s.c. (———) or 3 μg/g methamphetamine s.c. + 5 μg/g propranolol i.p. (– – – –). Mean values ± S.E.

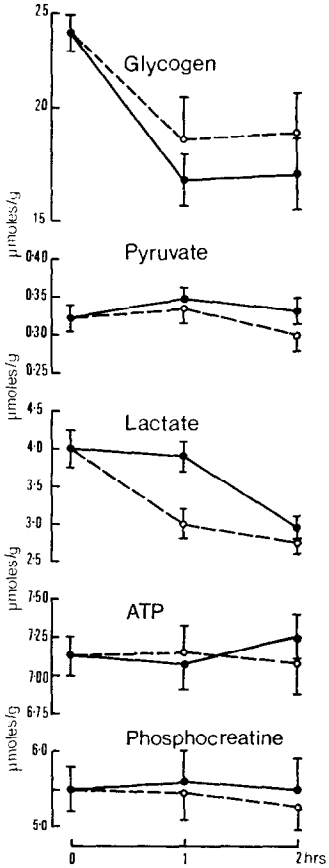

FIG. 3. Metabolite content of skeletal muscle of mice treated with 3 μg/g methamphetamine s.c. (———) or 3 μg/g methamphetamine s.c. + 5 μg/g propranolol i.p. (– – – –). Mean values ± S.E.

No changes occur in the pyruvate, ATP and phosphocreatine contents of the skeletal muscle (Fig. 3).

Methamphetamine + Propranolol

In the animals treated with methamphetamine (3 μg/g s.c.) together with propranolol (5 μg/g i.p.) the non-esterified fatty acids in the blood are lowered after 1 hr. They rise within the second hour of treatment and after 2 hr reach the same values as in those animals treated with methamphetamine alone. During the first hour the glycogen content of the liver falls lower than in the animals treated with methamphetamine alone but after 2 hr there is no difference between the two groups. There is no increase of the blood glucose level (Fig. 2). The glycogen content of the muscular tissue is reduced by almost the same amount as in the animals treated with methamphetamine alone. The small difference between the two treatments is not significant. The lactate content of the skeletal muscle shows a significant decline after 1 hr but no further decline during

the second hour. At the end of the second hour there is no significant difference in the lactate content of muscular tissue from mice treated with methamphetamine or methamphetamine + propranolol. The pyruvate, ATP and phosphocreatine contents of skeletal muscle remain constant during the course of the experiment (Fig. 3).

DISCUSSION

Methamphetamine

One of the most prominent effects of methamphetamine in mice is the several-fold increase of spontaneous motility. It is a matter of course that the muscular work, which is required for the increased locomotor activity, can be performed only if an adequate supply of substrates to the skeletal muscles especially of the legs, is ensured. Our results reveal, that in fact the substrate supply is enhanced by methamphetamine.

Methamphetamine raises the non-esterified fatty acid level in the blood. This is certainly the consequence of a stimulation of adipose tissue lipolysis as is indicated by analogous results obtained with amphetamine.[17,18] While the fatty acid level rises sharply during the first hour, a new equilibrium is reached in the second hour. Since uptake and oxidation of fatty acids in skeletal muscle are a function of the fatty acid concentration in the surrounding medium and since fatty acids are the main source of energy for working muscle,[19] an increased lipid mobilization and a rise of the fatty acid level in the blood is of special importance for the energy production in working skeletal muscle.

Furthermore, as is shown by the decrease of the glycogen content, methamphetamine, probably due to its sympathomimetic properties, mobilizes the glycogen stored in the liver. While the glycogen content of the liver falls continuously, glycogenolysis in skeletal muscle seems to come to a stand-still after 1 hr. Part of the hepatic glycogen is converted to glucose and released into the blood. The rise of the blood sugar level means, that more carbohydrates are made available to the extrahepatic organs including muscular tissue.

Since the decrease of the muscular glycogen is not accompanied by a corresponding increase of pyruvate and lactate, and since in the second hour, when glycogen breakdown in muscle is no longer accelerated, the lactate content falls even below the control values, one must assume that, in order to augment the energy yield, a greater percentage of pyruvate formed from the carbohydrates broken down is now transferred into the citric acid cycle and that less pyruvate is converted into lactate. Thus, a more extensive utilization of the carbohydrates may be accomplished.

That, in fact, in this way substrate supply and energy production in skeletal muscle are adjusted to the enhanced energy requirements is shown by the constant ATP and phosphocreatine contents. In conclusion, our results reveal the following integrated picture: methamphetamine drives the skeletal muscle to enhanced activity and at the same time provides enough substrate to meet the energetic needs.

Methamphetamine + propranolol

First hour. The most important effect of propranolol in our experiments is its antilipolytic action which prevents the methamphetamine induced rise of the non-esterified fatty acid level in the blood and even leads to a slight decrease of the non-esterified fatty acids. This means a serious reduction of substrate availability especially

since oxidation of fatty acids may normally cover up to 50 per cent of the energy production of working muscle.[19]

The changes observed in the carbohydrate metabolism must be interpreted as consequences of this primary effect of propranolol. The enhanced breakdown of liver glycogen is a compensatory mechanism, by which more carbohydrates are made available to extrahepatic organs. Similar observations have been reported with other antilipolytic drugs in resting and working subjects.[20-22] That, in spite of enhanced glycogenolysis in the liver, the blood glucose level does not rise significantly above the control levels and remains lower than in the animals treated with methamphetamine alone, may be explained by an increased uptake of glucose into various organs.

In contrast to the liver, the glycogen content of skeletal muscle is not significantly different from that in mice treated with methamphetamine alone. Obviously, unlike the higher doses of propranolol which markedly inhibit spontaneous and activated glycogenolysis in muscular tissue,[8,9] the lower dose of propranolol now used is not effective enough to inhibit methamphetamine-induced glycogenolysis in skeletal muscle. On the other hand, a further stimulation of glycogenolysis could not be observed, either. The only difference between animals treated with methamphetamine alone and mice treated with methamphetamine + propranolol is in the lactate content, which shows a significant decrease after 1 hr in the methamphetamine + propranolol treated mice, i.e. at the time when lipolysis is inhibited. A reasonable explanation is, that under this condition carbohydrates are utilized more quantitatively via the citric acid cycle and that less pyruvate is converted to lactate. This view, that less fatty acids are utilized in the methamphetamine + propranolol treated mice than in the animals treated with methamphetamine alone, is supported by the respiratory quotient of the animals. In methamphetamine-treated mice the RQ was 0·80 instead of 0·92 in the control group,[23] whereas the RQ of the animals treated with methamphetamine + propranolol was not significantly different from the controls.

By these measures the organism is able to provide enough energy for the skeletal muscle to maintain constant ATP and phosphocreatine contents, but on a lower activity level. Although it is unlikely, that propranolol produced an energy deficit, the results provide no conclusive evidence that substrate supply was really high enough during the first hour of treatment, to allow the animals to increase their muscular activity to the same level as the animals treated with methamphetamine alone.

Second hour. Obviously, the metabolic effects of propranolol are only shortlasting. In the second hour lipolysis is no longer inhibited and the fatty acid level reaches the same level in both groups. As consequence of the increased availability of fatty acids the glycogen breakdown in the liver can be slowed down, what is reflected also in the blood glucose level. After 2 hr no significant differences in the metabolite content of mice treated with methamphetamine or methamphetamine + propranolol are found.

Although there is no indication for substrate or energy deficiency at this time, the depressing effect of propranolol on methamphetamine-induced excitation persists. From these results the conclusion may be drawn, that the antagonism between propranolol and methamphetamine-excitation is not due to the peripheral metabolic but to the central nervous system effects of propranolol. This is in accordance with the results of other investigations from our laboratory, which show that the central antiadrenergic properties of propranolol may be responsible for its methamphetamine antagonistic action.[2]

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