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CHANGES IN CEREBRAL CARBOHYDRATE METABOLISM IN THE RAT AFTER ACUTE AND CHRONIC TREATMENT WITH, AND WITHDRAWAL OF, METHAMPHETAMINE

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Abstract—Groups of rats were treated with methamphetamine as follows: "acute", single intraperitoneal injection (5 mg/kg body wt); "chronic", receiving the drug in the drinking water in concentrations which increased from 5 mg/kg/24 hr initially to 40 mg/kg/24 hr after 3 weeks; "withdrawn", treated identically to the chronic group but given drug-free water for the final 24 hr before death. All groups, with controls, were injected with ^{14}C glucose at various times (5–15 min) before death by rapid freezing in liquid N_2. Changes observed in the brains of the acute group (increased levels of lactate, decreased levels of glycogen and increased rates of labelling of the glycogen) disappeared on chronic treatment and were reversed in the "withdrawn" group. It is suggested that the evidence is in favour of habituation on chronic treatment of methamphetamine and that the effects of withdrawal, similar as they were to those of a depressant drug, indicate that dependence has developed.

AMPHETAMINES were originally thought to give rise only to psychological dependence, but it has subsequently been realized that there is no ready distinction between physical and psychological dependence: part of the basis for amphetamine dependence may be physical.[1,2]

Biochemical studies on the effects of amphetamines have indicated interactions mediated by catecholamine release and antagonized by adrenergic α- and β-blocking agents.[3–5] One of the mechanisms by which the amphetamines act has been suggested to involve interference with catecholamine re-uptake.[6,7] Research on the effects of the β-blocker, propranolol, on methamphetamine action in mouse brain, has shown changes in levels of carbohydrate metabolites in the brain after acute treatment with methamphetamine. Decreases in glycogen and creatine phosphate were accompanied by increases in concentrations of glucose and of pyruvate; the rate of labelling of cerebral glycogen from injected [^{14}C]glucose also increased.[3,8]

This paper reports the effects of acute and chronic methamphetamine treatment, and of its withdrawal from chronically-treated rats, on levels and rates of turnover of selected intermediates of brain carbohydrate metabolism.

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MATERIALS AND METHODS

Treatment of animals

The experiments were performed on littermate Wistar rats of both sexes, 50–100 g body wt, and were treated as three main groups.

(1) "Acute", receiving intraperitoneally 5 mg methamphetamine HCl/kg body wt, made up as a solution (2 mg/ml) in 0·9% saline. They were killed by immersion in liquid N_2, 60 min later. Controls for this group were injected intraperitoneally with the equivalent volume of 0·9% saline.

(2) "Chronic", receiving methamphetamine in the drinking water in concentrations calculated to give 5 mg/kg/24 hr initially. This was doubled every 5 days to a final intake of 40 mg/kg/24 hr at the end of 3 weeks, when the rats were killed by rapid freezing. Controls had normal tap water throughout.

(3) "Withdrawn". This group was treated identically to the chronic group except that they were given drug-free water for 24 hr before death by rapid freezing. The control group was given tap water throughout.

All groups, treated and control, were given U-[^{14}C]-D-glucose (10 μCi/100 g body wt) intraperitoneally at various times (between 5 and 15 min, as noted) before death.

The cerebral cortex was removed from the frozen animals in a cryostat at $-25°$, and ground to a coarse powder in a mortar cooled in liquid N_2. After weighing in a metal scoop, they were dispersed by light hand-homogenization in glass–pestle homogenizers in 0·3 M perchloric acid (2 ml) at 0°. Each value is the mean of duplicate tissue samples.

After centrifugation at 3000 rev/min for 30 min at 0°, the pellets were retained for glycogen estimation and the supernatants were neutralized with 3 M-$KHCO_3$ at 0°, centrifuged to remove precipitated $KClO_4$ and used for estimations of acid-soluble metabolites.

Metabolite estimations

Glycogen was partially purified by extracting the perchloric acid pellet into hot water (100° for 10 min). After cooling to 0° and centrifugation, the glycogen was estimated enzymically using amylo α 1, 4-α 1, 6 transglucosidase.[9]

Glucose, *lactate* and *glutamate* were estimated by methods described previously.[10]

Specific activities of the metabolites were estimated as described previously.[10]

Reagents

Methamphetamine–HCl was purchased from Sigma (London) Chemical Co., Ltd. All enzymes and coenzymes used in the estimations were supplied by Boehringer Corp., London.

D-Glucose-^{14}C(U) (3 mCi/m-mole) was obtained from the Radiochemical Centre, Amersham.

RESULTS

Effects of methamphetamine on metabolite concentrations. A single dose of methamphetamine (5 mg/kg) resulted 1 hr later in a decrease in glycogen of 18 per cent and an increase in lactate of 13 per cent (Table 1). There was no significant change in cerebral concentration of glucose or of glutamate. The changes in lactate and gly-

TABLE 1. CHANGES IN CONCENTRATION OF CEREBRAL METABOLITES AFTER TREATMENT WITH METHAMPHETAMINE AS DESCRIBED IN METHODS

Metabolite	Control value ($n = 24$) (μmole/g $\pm$ S.D.)	Change (as % of control value)		
		Acute	Chronic	Withdrawn
Glucose	1·58 $\pm$ 0·34	107 (NS)	94 (NS)	119 ($P < 0·025$)
Lactate	1·93 $\pm$ 2·28	113 ($P < 0·005$)	101 (NS)	85 ($P < 0·005$)
Glutamate	11·88 $\pm$ 2·01	103 (NS)	96 (NS)	96 (NS)
Glycogen	1·43 $\pm$ 0·15	82 ($P < 0·0005$)	102 (NS)	123 ($P < 0·025$)

cogen, though highly significant, were small in contrast to the effects on the rate of labelling from [^{14}C]glucose, described below. These changes, observed in the "acute" experiments, were not observed after chronic treatment when no significant differences could be observed. On the other hand, when the methamphetamine was withdrawn from the chronically-treated animals, the changes observed were the complete opposite of those noted after acute treatment: glycogen increased by 22 per cent and lactate decreased by 15 per cent (Table 1). There was no change in glutamate and a 19 per cent increase in glucose.

Incorporation of ^{14}C from glucose into cerebral metabolites. The most striking results were in the rate of labelling of glycogen (Fig. 1). Thus in the acute experiments (Fig. 1a), methamphetamine treatment resulted in a significant increase in the rate of incorporation of ^{14}C into glycogen, significantly different ($P < 0·005$) at times from 9 min on. At 9 min the specific activity of the brain glycogen was twice the control value and the total ^{14}C present in the glycogen was 235 per cent of that in the control animals. There was no effect on glycogen labelling after chronic treatment, but on withdrawal, the rate of labelling of the glycogen was significantly decreased (Fig. 1b). The specific activity of the glycogen at 9 min was 50 per cent of the control value and the total ^{14}C in the glycogen had decreased by 30 per cent. The specific

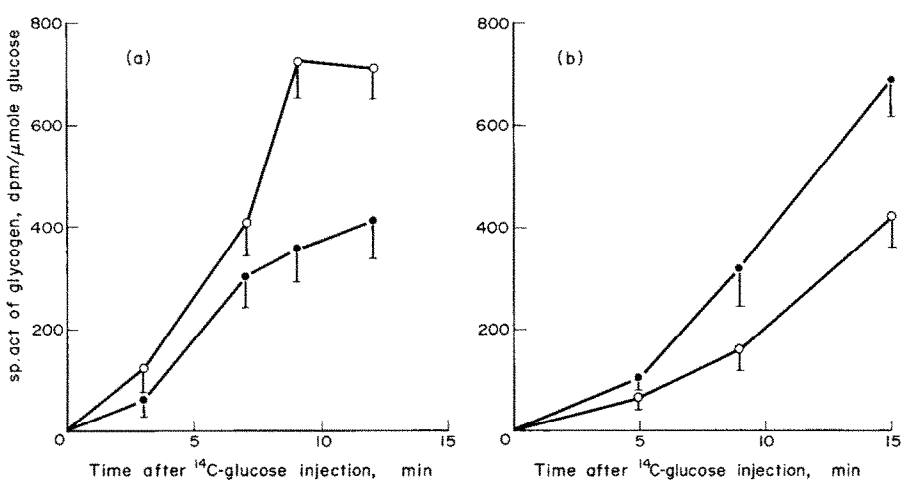

FIG. 1. Incorporation of ^{14}C from glucose into glycogen, in methamphetamine-treated rats: (●) control; (○) treated; (a) "acute" experiments; (b) "withdrawal". Each value is the mean of the results from at least four rats. The vertical bars represent S.D.

activity of cerebral lactate at 9 min after injection of ^{14}C glucose was not significantly different after acute or chronic treatment, but was 27 per cent lower after withdrawal, compared with control values. There was no effect on glutamate in any of the experimental groups. Thus an opposite effect was also observed in the labelling of glycogen between acute treatment and withdrawal, and the pattern of labelling of the metabolites on withdrawal was again similar to that seen in the presence of a depressant drug; a general decrease in the rate of labelling of metabolites (glycogen and lactate) derived from glucose.

DISCUSSION

The results on tissue concentrations of glycogen, lactate and glutamate after the acute treatment with methamphetamine are similar to those observed after similar treatment with *dl*-amphetamine. The effects on brain glycogen are also in agreement with the results reported by Estler and Ammon[3] and by Estler and Mitznegg,[8] who suggested that the increase in specific activity might be due to increased turnover rates resulting from increased breakdown of glycogen by catecholamine activation of phosphorylase. The doubling of the specific activity of the glycogen at 9 min in the acute experiments of the present study was equivalent to an increase to 235 per cent of the control value for the total ^{14}C present in the glycogen, and the rate of incorporation of ^{14}C into glycogen was faster over the entire experimental period after acute treatment with the drug. Calculations of approximate turnover rates, from the rate of change of radioactivity in glycogen and lactate, indicated that the turnover rate of the glycogen was only some 5 per cent of that of the lactate in both acute and control groups and is a value similar to that previously found.[11] In that case the observed decrease in glycogen of 0·25 μmole/g would lead to an accumulation of no more than 0·025 μmoles of lactate, if as seems probable from our results, there is no significant effect on metabolism of lactate through the tricarboxylic acid cycle to glutamate. However the increase in lactate was 0·25 μmole/g, and only 10 per cent of this is likely to have been derived directly from glycogen. The increase in lactate may therefore be due more to increased rates of glycolysis from glucose rather than from glycogen alone. Amphetamines have been reported to inhibit, rather than activate, cerebral phosphorylase.[12] The relatively small increase in blood glucose concentration after treatment with methamphetamine[3] is unlikely to cause in itself, pronounced stimulation of glucose metabolism[13] unless the drug exerts a direct effect on glucose transport.

Chronic treatment and withdrawal. The disappearance of any stimulation of cerebral metabolism after chronic treatment by administering the amphetamine in the drinking water could have resulted from lack of intake of the drug, to lack of its penetration to the brain, or it could indicate habituation. It seems unlikely to have been due to changes in breakdown or excretion of the drug.[14] The water intake of the chronically treated animals was only slightly (18 per cent) lower than of the controls, so lack of intake of the drug can be eliminated. We believe that the animals had become habituated to the drug, which receives support from the observed effects of withdrawal. There were in direct contrast to the results from the acute experiments, and were of a pattern more similar to those seen after treatment with depressant drugs (e.g. barbiturates[11,15]). The decrease in glycolytic rates is compatible with the decreased respiration seen to occur on withdrawal of amphetamines.[16] Although

rigorous behavioural tests were not performed in this study, the observed behaviour of the animals seemed consistent with the metabolic results: the hyperactivity, aggression and stereotypy of the acute group was not obvious after chronic treatment; the withdrawn group were docile.

We conclude that these studies form a basis for developing animal models for dependence on amphetamine since the stimulation effects on cerebral metabolism after acute treatment disappear on chronic administration of the drug and are reversed after its withdrawal.

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REFERENCES

1. W.H.O. Expert Committee, *Tech. Rep. Ser. Wld Hlth. Org.* **116,** 9 (1957).
2. W.H.O. Expert Committee, *Tech. Rep. Ser. Wld. Hlth. Org.* **273,** 10 (1964).
3. C.-J. Estler and H. P. T. Ammon, *J. Neurochem.* **14,** 799 (1967).
4. J. Cahn and M. Herold, in *Amphetamines and Related Compounds* (Eds. E. Costa and S. Garattini) p. 493. Raven Press, New York (1970).
5. P. Mantegazza, E. E. Muller, M. K. Naimzada and M. Riva, in *Amphetamines and Related Compounds* (Eds. E. Costa and S. Garattini) p. 559. Raven Press, New York (1970).
6. A. Carlsson, in *Abuse of Central Stimulants* (Eds. F. Sjoqvist and M. Tottie) pp. 305–310. Almqvist & Wiksell, Stockholm (1969).
7. L. L. Iversen, in *Adrenergic Neurotransmission* (CIBA Study Group No. 33) pp. 44–56. Churchill, London (1968).
8. C.-J. Estler and P. Mitznegg, *Biochem. Pharmac.* **20,** 1331 (1971).
9. H. S. Bachelard and R. H. C. Strang, in *Research Methods in Neurochemistry* (Eds. N. Marks and R. Rodnight) Vol. 2. Plenum Press, New York (1973).
10. R. H. C. Strang and H. S. Bachelard, *Analyt. Biochem.* **41,** 533(1971).
11. R. H. C. Strang and H. S. Bachelard, *J. Neurochem.* **20,** 987 (1973).
12. T. T. Iriye, A. Kuna and F. Simmonds, *Biochem. Pharmac.* **11,** 803 (1962).
13. H. S. Bachelard, P. M. Daniel, E. R. Love and O. E. Pratt, *Proc. r. Soc., Lond.* **B183,** 71 (1973).
14. T. Lewander, *Psychopharmacologia* **13,** 394 (1968).
15. P. D. Gatfield, O. H. Lowry, D. W. Schulz and J. V. Passonneau, *J. Neurochem.* **13,** 185 (1966).
16. H. Utena, T. Ezoe, T. Kato and H. Hada, *J. Neurochem.* **4,** 161 (1959).