Acta Chemica Scandinavica B 32 (1978) 322—326

Stimulation of L-Ornithine and S-Adenosyl-L-methionine Decarboxylases by β-(p-Chlorophenyl)-γ-aminobutyric Acid in Mouse Tissues

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Injections of β-(p-chlorophenyl)-γ-aminobutyric acid caused a monophasic stimulation of the activity of neural L-ornithine decarboxylase, to reach a maximum of 9-fold compared with the control values 3 h after treatment. Stimulation of hepatic L-ornithine decarboxylase was biphasic, the activity reaching its first peak, 48-fold compared with the control values, similarly at about 3 h after administration, and returning to its initial level by 4 h, and rising to a second peak, about one third of the magnitude of the first, about 25 h after the injection. The effect in the adrenal gland of the mouse was multiphasic, reaching its maximum, 94-fold enzyme activity compared with the control values, 7 – 8 h after treatment. There were also marked fluctuations in the activity of S-adenosyl-L-methionine decarboxylase in the tissues examined.

Many reports suggest that polyamines are involved in cell growth and that the rate-limiting enzyme for their synthesis is L-ornithine decarboxylase [1–3] (L-ornithine carboxy-lyase, EC 4.1.1.17). The polyamines have been shown to affect transcription, translation and the methylation and aminoacylation of tRNA.[3] Thus a dramatic increase in the activity of L-ornithine decarboxylase (ODC) is one of the earliest detectable events in rapidly growing tissues such as regenerating rat liver,[3–5] developing embryos [6,7] and certain tumours.[8] S-Adenosyl-L-methionine decarboxylase (S-adenosyl-L-methionine carboxy-lyase, EC 4.1.1.50) also shows changes in activity, but these are less marked. The activity of ODC is also markedly elevated in the liver after treatment with drugs known to produce liver enlargement upon acute or chronic administration, such as thioacetamide,[9–12] phenobarbital [13] or 3-methylcholanthrene.[13] It has also been shown in earlier studies that electric shock treatment stimulates ODC and S-adenosyl-L-methionine decarboxylase (SAM-DC) to a marked extent in adult mouse brain,[14] suggesting that polyamines may have another function in addition to their involvement in cell growth.

The present work investigates the effect of another central nervous system-active factor, β-(p-chlorophenyl)-γ-aminobutyric acid (β-p-CPG), on the activities of ODC and SAM-DC in adult mouse brain. β-p-CPG is a lipophilic derivative of γ-aminobutyric acid, which probably is an inhibitory transmitter in the vertebrate central nervous system. This derivative has been reported to be of clinical value in the management of spasticity.[15] Its mode of action is not yet known, but it has been suggested that it may act on certain receptors for inhibitory transmitters at the neuronal membrane which are chemically related to phenylethylamine.[16] The effects of β-p-CPG on the activities of these enzymes were also studied in adult mouse liver and adrenal gland as reference tissues.

MATERIALS AND METHODS

Chemicals. Unlabelled S-adenosyl-L-methionine was synthetized by the method originally described by Cantoni and Durrel [17], as modified by Pegg and Williams-Ashman.[18] (Carboxyl-^{14}C)-S-adenosyl-L-methionine (specific radioactivity 54.6 mCi/mmol) was purchased from New

England Nuclear Corp. (Boston, Mass., U.S.A.) and DL-ornithine-1-^{14}C (specific radioactivity 59 mCi/mmol) from the Radiochemical Centre (Amersham, Bucks., U.K.). DL-Ornithine-1-^{14}C was treated before use as described previously.[19] Putrescine was obtained from Calbiochem (San Diego, Calif., U.S.A.). β-(*p*-Chlorophenyl)-γ-aminobutyric acid (baclofen; Lioresal®), kindly supplied by Ciba-Geigy (Basle, Switzerland), was dissolved in 0.9 % NaCl solution just before injection. All other chemicals were of reagent grade.

Animals. Adult male mice of the Swiss Albino strain weighing about 30 g were used in all the experiments. These were kept in regularly alternating 12 h periods of light and dark and allowed food (standard pelleted diet: Hankkija Oy, Turku, Finland) and drink *ad libitum*. All animals were killed by decapitation between 10.00 and 12.00 a.m. to eliminate the influence of diurnal variations in enzyme activities.

Assay methods. The brain, liver and adrenal gland were removed immediately and homogenized with 3 volumes of cold 25 mM Tris-HCl buffer, pH 7.4, containing 0.1 mM EDTA, 5 mM dithiothreitol and 0.1 mM pyridoxal 5'-phosphate. The homogenates were centrifuged at 105 000 g_{max} for 45 min at 4 °C. The enzyme activities were assayed immediately using undialyzed supernatant fractions as the enzyme source. The assay conditions for ODC were essentially as described by Jänne and Williams-Ashman[19] except that 4 mM EDTA was used in the reaction mixture to lower the otherwise high blank values and 0.1 M Tris-HCl buffer was used. The activity of SAM-DC was measured by the method of Jänne and Williams-Ashman.[20] The enzyme activities are expressed as pmol of product formed per mg protein per 30 min.

Protein concentrations were determined with bovine serum albumin as standard.[21]

RESULTS

Effect of β-p-CPG on polyamine-synthesizing enzymes in mouse brain. The changes in enzyme activities in mouse brain after treatment with β-*p*-CPG are shown in Fig. 1. When injected intraperitoneally at a dose of 45 μmol/kg body weight the compound stimulated the neural ODC about 9-fold, the peak occurring 3 h after treatment. A moderate decrease was noted in SAM-DC activity, reaching a minimum at the same time as the ODC maximum. At this point the level of enzyme activity in the brain was about 91 % of that in the control animals. A peak in enzyme activity extending above the control level was found after 20 h, followed by a decline. SAM-DC activity regained its control level after 45 h.

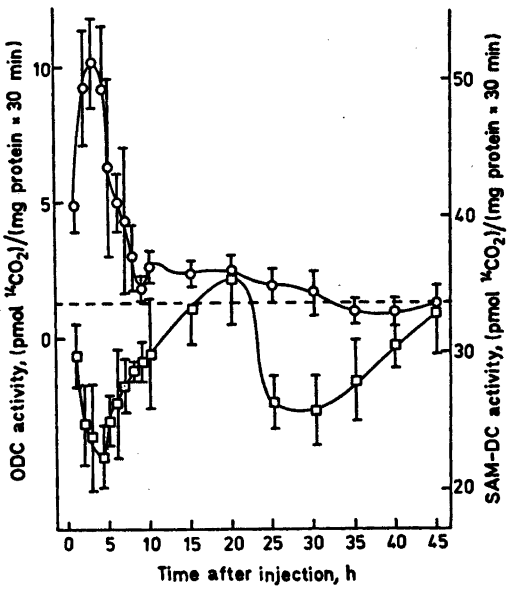

Fig. 1. Effect of β-*p*-CPG on ODC and SAM-DC activities in mouse brain. The mice received an intraperitoneal injection of β-*p*-CPG, 45 μmol/kg. The enzyme activities were measured as described in the text. Each point is the mean of 6 determinations, with vertical bars representing standard deviations. ○, ODC activity, □, SAM-DC activity. Broken line denotes control levels of both enzymes. Standard deviation for ODC ± 0.62 and SAM-DC ± 2.93.

Effect of β-p-CPG on polyamine-synthesizing enzymes in mouse liver. The changes in enzyme activities in mouse liver after treatment with β-*p*-CPG are shown in Fig. 2. A biphasic stimulation in the activity of hepatic ODC was observed, with the maximum, 48-fold compared with the control values, being reached after about 3 h administration, a fall to the initial values after 4 h, and a second peak, 16-fold, after about 25 h.

SAM-DC activity tended to decrease in the β-*p*-CPG-treated animals. After 1 h the level of enzyme activity in liver was about 16 % of that in the control animals. The fluctuations resembled those found in ODC, but occurred somewhat later and were less pronounced.

Effect of β-p-CPG on polyamine-synthesizing enzymes in mouse adrenal gland. The changes in enzyme activities in mouse adrenal gland

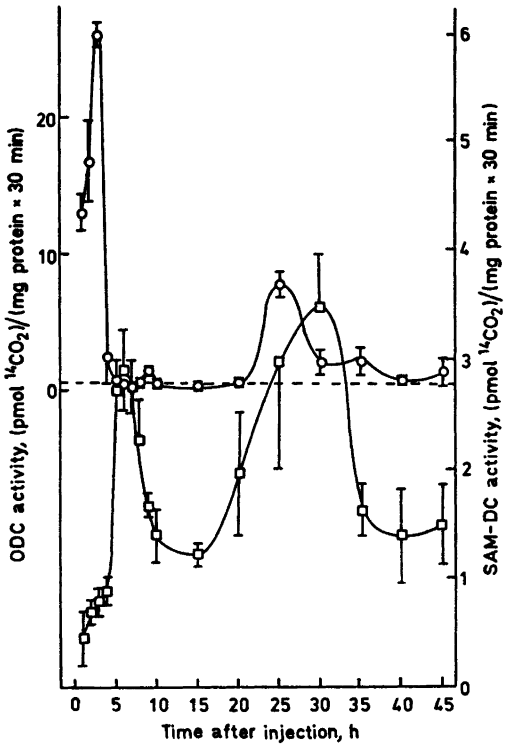

Fig. 2. Effect of β-*p*-CPG on ODC and SAM-DC activities in mouse liver. The animals were treated with β-*p*-CPG as described in Fig. 1. Each point is the mean of 6 determinations, with vertical bars representing standard deviations. ○, ODC activity, □, SAM-DC activity. Broken line denotes control levels of both enzymes. Standard deviation for ODC ± 1.19 and SAM-DC ± 0.61.

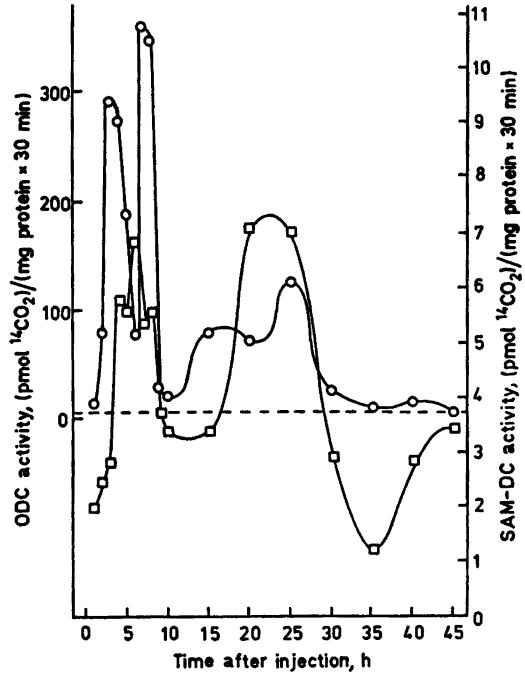

Fig. 3. Effect of β-*p*-CPG on ODC and SAM-DC activities in mouse adrenal gland. The administration and dose of β-*p*-CPG were as described in Fig. 1. Each point represents the mean of two pooled samples, each obtained from ten adrenal glands. ○, ODC activity, □, SAM-DC activity. Broken line denotes control levels of both enzymes.

after treatment with β-*p*-CPG are shown in Fig. 3. The injections caused a multiphasic stimulation in the activity of adrenal ODC, which increased to 75-fold compared with the control values 3 – 4 h after the injection, fell sharply, reached another peak, 94-fold, 7 – 8 h after treatment, and then decreased to close to the control values within 9 – 10 h. Further minor peaks occurred after 15 and 25 h.

SAM-DC activity decreased at first, being about 54 % of that observed in the control animals at 1 h, but was then stimulated in two phases having a time-course similar to that observed with ODC, although the fluctuations were less pronounced (Fig. 3).

DISCUSSION

Since an increase in the biosynthesis and accumulation of polyamines is always associated with rapid growth,[22,23] the aim of the present work was to compare the induction of polyamine-synthesizing enzymes in the mouse in three tissues, one of which is known to be nonproliferous. β-*p*-CPG, which is able to cross the blood-brain barrier,[24] stimulated ODC in all the tissues examined. Major elevations were observed in the adrenal gland, to a maximum of 94 times the normal, a 48-fold rise in activity occurred in the liver during the first few hours and a 9-fold increase was found in the brain after 3 h.

Variations in the extent and timing of SAM-DC activation were also noted, the increases in activity occurring later than in the

case of ODC in the liver and brain. It is significant that the activities of ODC and SAM-DC changed in the opposite directions during the period 3 – 7 h in all the tissues examined, a feature which has also been observed in mouse brain after electric shock treatment,[14] but remains unexplained. ODC has been reported to undergo a 10 – 40-fold rise in activity in regenerating rat liver during the first few hours after partial hepatectomy,[2] and Hölttä and Jänne [5] even obtain a biphasic pattern of activation similar to that found here, even though the first peak of activity consistently appeared only 4 h after the operation, whereas the induction of ODC caused here by β-*p*-CPG administration was noticeable 1 h after treatment and reached its peak after 3 h. Schrock *et al.*[25] and Fausto [26] report that various unspecific substances such as casein hydrolyzate, histidine and Celite injected intraperitoneally induce ODC in rat liver, and they similarly observe an early stimulation in enzyme activity. It is possible that γ-aminobutyric acid itself, like these unspecific substances, would also induce hepatic ODC, but because of the low permeability of the blood-brain barrier for this substance it is probable that no induction would occur in the brain. It is not known, however, whether these substances, including β-*p*-CPG, in the liver primarily enhance the enzyme activity or just result in a release of growth hormone or corticosteroid which is secondarily responsible for the stimulation. It is also unclear whether the stimulation of ODC activity in the liver is a specific indicator of liver growth, especially in the presence of the pituitary or adrenals. The timing of the second peak of ODC activity in the liver probably depends on the age of the animal.[5]

The dramatic increase in adrenal ODC activity after β-*p*-CPG administration supports the suggestion that catecholamines are involved in the antispastic effects produced by β-*p*-CPG.[27] Both exposure to cold and the administration of aminophylline result in rapid increases in cyclic adenosine monophosphate (cAMP), followed by dramatic rises in ODC activity in the adrenal medulla and adrenal cortex, suggesting that the decarboxylase activity is regulated by an increase in cAMP.[28,29] However, the induction of ODC and the increased accumulation of cAMP are probably not causally related, since the stimulation of adenyl cyclase in regenerating rat liver after partial hepatectomy can be prevented by β-adrenergic blocking agents without any influence on the activity of hepatic ODC. The induction of ODC, on the other hand, is prevented by a prior administration of α-adrenergic blocking agents.[30] It still remains to be established whether the action of β-*p*-CPG on the induction of ODC is mediated by cAMP through the activation of protein kinase.

Very little is known about polyamine-synthesizing enzymes in the mature brain. Complete cerebral ischemia and subsequent recirculation has been shown to produce an induction of ODC and SAM-DC activities in the brain of adult rhesus monkeys.[31] On the other hand, electric shock treatment also stimulates the activities of these enzymes in adult mouse brain.[14] Since the latter primarily stimulates the adrenal gland,[32] it seems likely that the induction of ODC and SAM-DC in this case, as in that of β-*p*-CPG, occurs via catecholamines.

Acknowledgement. We wish to thank Dr. J. Jänne for his helpful suggestions during the course of this work and for his critical review of the manuscript.

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Received December 27, 1977.