The ischemic and postischemic effect on the uptake of neutral amino acids in isolated cerebral capillaries

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Summary. The uptake of some neutral amino acids was investigated in cerebral microvessels isolated from brains of gerbils subjected to bilateral cerebral ischemia with and without various periods of recovery. A transiently increased capillary uptake of ^{3}H-isoleucine, ^{14}C-cycloleucine and ^{3}H-phenylalanine was found in both conditions.

The isolated cerebral microvessels have been useful in the investigation of transport processes occurring on the blood brain level. Recently we have shown that ischemia and anoxia reduce the capillary ^{3}H 2-DG uptake which could be recovered either by reestablishment of cerebral blood circulation or by substituting nitrogen with oxygen atmosphere[3,4]. The purpose of this investigation has been to evaluate the effect of cerebral ischemia and postischemia on the capillary uptake of substances which are transported across the blood brain barrier (BBB) by a specific carrier mediated process other than the one for hexose[5-7]. In this communication we will describe a transient ischemic and postischemic increase in the capillary uptake of some neutral amio acids.

Cerebral ischemia and postischemia were produced in gerbils by bilateral common carotid artery occlusion (1-30 min) and clip release for 1-120 min after 3- or 6-min arterial clipping. The cerebral capillaries were separated from the nonvascular tissue by centrifugation and sucrose gradient according to the previously reported technique but the concentration of sucrose gradient was changed from 1-1.5 M to 1-1.8 M, greatly improving the sample's purity[2]. The procedures for the determination of ^{3}H- and/or ^{14}C-labeled neutral amino acids were the same as one used for the uptake of 2-deoxy-D-[^{3}H]glucose except for the incubation of 2 instead of 15 min[2,3]. Isoleucine, L-[4,5-^{3}H(N)] (spec. act. 80.5 or 103.5 Ci/mM), aminocyclopentane-1-carboxylic acid 1-[carboxyl-^{14}C] (spec. act. 29.9 mCi/mM), phenylalanine [^{14}C(U)] and phenylalanine L-[alanine-3-^{3}H(N)] (spec. act. 464 mCi and 21.6 Ci/mM, respectively) and glutamine L-[^{14}C(U)] (spec. act. 200 mCi/mM) were purchased from New England Nuclear Co., Boston, Mass. The unlabeled (cold) amino acids used for the nonspecific uptake and the inhibition studies were obtained from Sigma Chemical Co., St. Louis, Mo.

The cerebral capillaries isolated from brains of gerbils subjected to bilateral common carotid artery occlusion for 1-30 min duration showed a transiently increased uptake of isoleucine, cycloleucine and phenylalanine at 3 min (figure 1), as previously observed in synaptosomes. The capillary glutamine uptake was not affected by cerebral ischemia. The increased capillary amino acid uptake dropped to a lower level at 1 min and remained significantly higher than the one of controls up to 10 min but returned to normal values after reestablishment of cerebral blood circulation. Moreover, an augmented uptake of these amino acids was observed in microvessels separated from brains of animals subjected to 6 min of bilateral carotid artery occlusion and 1-30 min release. 2 h later the capillary uptake of tested amino acids returned to normal levels (figure 1). In both circumstances the increased isoleucine and phenylalanine uptake could be inhibited by various concentrations of cold cycloleucine or phenylalanine to the same degree as in controls, respectively (figure 2). Although the postischemic effect on the amino acid uptake is similar to the one described in the capillary 2-DG but the 6 min occlusion resulted in normal entry of the amino acids while a reduction of 2-DG uptake was seen in the cerebral capillaries at the same time[3].

These results indicate that the ischemic and postischemic

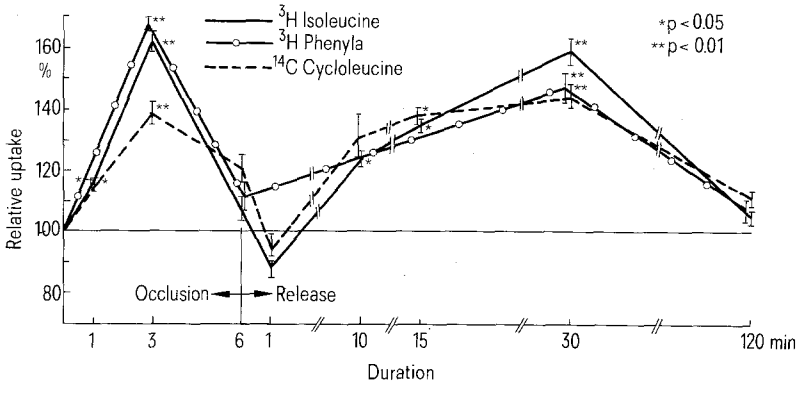

Fig. 1. The neutral amino acids uptake in capillaries separated from brains of gerbils subjected to bilateral common carotid artery clipping for 1-6 min and 1-120-min clip release after 6 min of occlusion. Duplicate aliquots of the isolated capillaries (0.1 ml) were incubated in 0.5 ml Ringer-albumin solution containing one of the isotopes (pH 7.4). The final concentration of ^{3}H-isoleucine, ^{3}H-phenylalinine and ^{14}C-cycloleucine was 2.18×10^{7}, 6.4×10^{7} and 2.1×10^{3} cpm/min/μmole respectively. The concentration of total capillary protein in all experimental groups was similar to the controls. Each point represents the mean ±SEM of 6-12 experiments.

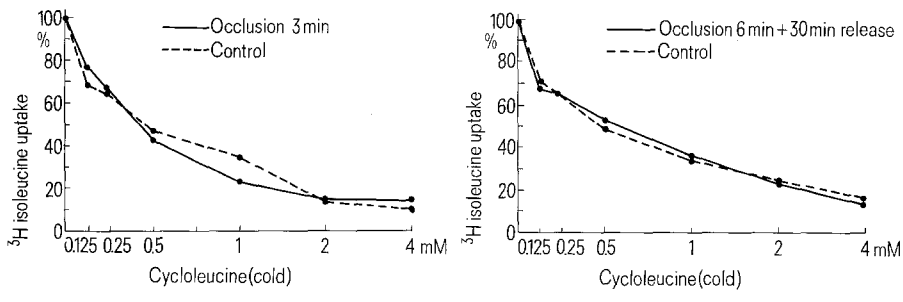

Fig. 2. Inhibition of ^{3}H-isoleucine uptake with various concentrations of unlabeled cycloleucine in capillaries obtained from brains of experimental and control gerbils. Each point is an average of 4 duplicate determinations with variations of less than 5%.

augmented entry of the amino acid is the result of an increased transport rather than metabolism since a similar increase in the uptake of the metabolizable amino acid isoleucine as of the nonmetabolizable analogue cycloleucine was observed in the isolated microvessels. The complete inhibition of the increased isoleucine uptake by the cold cycloleucine supports this contention and indicates the involvement of the carrier mediated process. Hence, the increased uptake of phenylalanine could also be due to the specific passage and not metabolism. It is of interest that such a change was not seen in the capillary uptake of glutamine which belongs to the substances characteristic for low level passage across the BBB[5-7]. However, a similar increase in the specific uptake of these amino acids was seen in the cerebral capillaries exposed to a medium containing NaCl under anaerobic conditions only (unpublished observations). Therefore, it is possible that the ischemic and postischemic increased capillary uptake of the labeled isoleucine, cycloleucine and phenylalanine may be due to ionic changes and altered reactivity of the carrier mediated process for these substances.

In conclusion, selective and diverse effects of cerebral ischemia and postischemia on the capillary uptake of the neutral amino acids and on the previously reported entry of ^{3}H 2-DG[3,4] could be responsible for the different passage of these substances across the BBB under these conditions.

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Nerve-growth promoting action of isaxonine in rat

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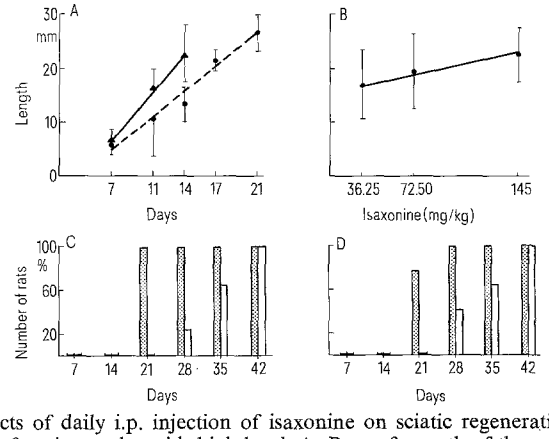
Summary. After sciatic nerve lesion by freezing, the length of the most rapidly regenerating fibres was significantly increased by i.p. injection of isaxonine (N-isopropyl-amino-2-pyrimidine orthophosphate) in the rat. A dose-effect relationship was demonstrated. Both sensory and motor function returned earlier in treated animals.

After nerve injury, the process of nerve regeneration is slow. It may be possible that drugs which accelerate nerve growth could be useful in the clinical treatment of nerve injuries, as well as a tool for investigating mechanisms controlling nerve growth and regeneration. We reported recently[1] that isaxonine[2] (N-isopropyl-amino-2-pyrimidine orthophosphate), a newly synthesized drug, promotes a powerful neurite outgrowth in the cultured spinal ganglion of the mouse. The present experiments examine the possible nerve growth promoting action of isaxonine in vivo in rat.

We compared the rate of regeneration of the sciatic nerve in control animals (Wistar males 180±5 g) with that of animals receiving isaxonine. The sciatic nerve was frozen at the mid-thigh level with a thermode 2 mm in diameter, maintained at −20 °C for 20 min[3]. This technique produces standard axon damage and wallerian degeneration. It does not disrupt the nerve sheath, leading to a more homogeneous rate of axon growth[4,5]. The length of regenerated fibres was measured electrophysiologically in a nerve chamber[6]. The sciatic nerve was excised under nembutal anaesthesia and immersed in paraffin oil at 37.5 °C in the chamber containing a grid of platinum electrodes 1 mm apart. The extremity of the most rapidly growing fibres was determined by measuring the distance between nerve injury and the most distal pair of electrodes from which an action potential could be detected after 12 summations. A computer-assisted technique made possible the sequential recording from 12 pairs of electrodes surrounding the extremity of regenerating fibres within 90 sec: this delay is shorter than the time required for alteration of action potential in the regenerating nerve after excision[3].

Measurements were carried out on groups of 10 animals 7, 11, 14, 17 and 21 days after the lesion (figure A). One series of treated animals received 145 mg of isaxonine (LD 50/10) i.p. each day. The rate of regeneration calculated from the slope of the regression line was 1.6 (±1.28 SE) mm/day in the control group and 2.3 (±1.17 SE) mm/day in isaxonine treated animals (+44%). Covariance analysis using the



Effects of daily i.p. injection of isaxonine on sciatic regeneration after freezing at the mid-thigh level. A: Rate of growth of the most rapidly regenerating axons as demonstrated by recording of action potential in a nerve chamber. Experimental points show the mean ±SD of the length from the lesion to the most distal electrode from which action potential could be recorded as a function of the postoperative day. The mean recovery rate is calculated by taking the reciprocal of the slope of the regression line. Dottet line = controls; solid line = animals treated with isaxonine 145 mg/kg daily (LD 50/10). B: Dose-effect regression line: length ±SD of the most rapidly regenerating axons 14 days after the lesion as a function of drug concentration. C and D: Effects of isaxonine (145 mg/kg daily) on the return of sensory (C) and motor (D) functions; striped bars = isaxonine-treated animals; white bars = controls injected with saline.