Life Sciences, Vol. 33, Sup. I, 1983, pp. 763-768 Pergamon Press
Printed in the U.S.A.

STUDIES OF NUCLEAR MAGNETIC RESONANCE IMAGING AND REGIONAL CEREBRAL GLUCOSE METABOLISM IN ACUTE CEREBRAL ISCHEMIA: POSSIBLE MECHANISM OF OPIATE ANTAGONIST THERAPEUTIC ACTIVITY

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(Received in final form June 26, 1983)

Summary

Using unilateral carotid artery ligation in the gerbil as a model of cerebral ischemia, both nuclear magnetic resonance (NMR) imaging and a newly developed double-label autoradiographic technique have been employed to investigate the physiologic mechanism of opiate action during cerebral ischemia. While several parameters of the NMR image have been demonstrated to reflect focal cerebral ischemic lesions, neither 2 mg/kg naloxone nor 10 mg/kg morphine sulfate had an effect on any of the parameters of the NMR image at any time point during the 24 hr experiment. While no consistent changes could be measured in the metabolic rate immediately about the ischemic region, results from double-label 2-deoxyglucose autoradiographic studies indicate that there are marked focal increases in metabolic rate in several subcortical nuclei bilaterally following the administration of naloxone. While no significant change was noted in the thalamus or arcuate nucleus, naloxone produced a significant elevation in glucose metabolic rate in the substantia nigra, periaqueductal grey and the red nucleus. The significance of these effects are discussed and a mechanism for the beneficial effect of opiate antagonists on neurologic deficit following ischemic cerebral lesions is proposed.

Several recent studies have reported a significant effect of opiate antagonists in reversing the neurologic deficits associated with ischemic cerebral insults. Baskin and Hosobuchi (1) first observed that the intravenous administration of naloxone, an opiate receptor antagonist, reversed much of the neurologic deficit seen in two patients with symptoms referable to cerebral ischemia. Naloxone subsequently has been shown to reverse the ischemic neurologic deficit in gerbils following unilateral carotid artery ligation (2) and in both baboons (3) and cats (4) after middle cerebral artery ligation. In a parallel study, Hosobuchi, et al., (2) have demonstrated that morphine can precipitate neurologic deficits in previously asymptomatic gerbils following unilateral carotid artery ligation. A central question in the interpretation of the beneficial effects of opiate antagonists on the neurologic deficits associated with cerebral ischemia is whether these agents act on the ischemic or nearly ischemic regions of the brain to restore circulation, decrease edema or improve metabolic activity; or alternatively whether they act upon brainstem motor nuclei to allow better control of motor function in the continued absence of normally functioning cortical input. A combination of these effects is, of course, also possible. Using unilateral carotid artery ligation in the gerbil as a model of acute

0024-3205/83 $3.00 + .00

cerebral ischemia, we have employed both nuclear magnetic resonance (NMR) imaging and a newly developed double-label 2-deoxyglucose autoradiographic technique to further evaluate the possible mechanism of opiate action in cerebral ischemia.

Methods

In the first set of experiments, 132 adult male gerbils weighing 55-70 g were anesthetized with 40 mg/kg pentobarbital intraperitoneally (I.P.). With the aid of the operating microscope, the right common carotid artery was isolated, coagulated with microbipolar forceps and divided. The wound was then closed and the animals allowed to recover from anesthesia. Three hours after surgery, animals were evaluated for neurologic deficit by the method of Hosobuchi, et al. (2).

Gerbils were divided into two groups: (1) those that were asymptomatic and (2) those demonstrating clear neurologic deficts. At three time points, ranging from 3-24 hr after carotid occlusion, groups of animals again were anesthetized with pentobarbital and NMR images were obtained according to the technique of Crooks, et al. (5). Following the initial imaging procedure, the effect of pharmacologic agents on the NMR image was examined. Symptomatic animals were given 2 mg/kg naloxone I.P. immediately following imaging at all three time points. Six to 10 min following naloxone administration, animals underwent repeat imaging. Following the initial imaging, asymptomatic animals were given 10 mg/kg morphine sulfate I.P. These animals underwent repeat imaging 60-90 min following morphine administration.

Each gerbil underwent NMR imaging at a time point 3-7, 12-16 or 24-28 hr following carotid occlusion. Animals were wrapped loosely in plastic sheeting for immobilization and placed in a supine position in the imaging field. The gerbil brain was imaged with the coronal plane bisecting both hemispheres symmetrically. The NMR imager used for this study has been described in detail elsewhere (5,6). It has a resistive magnet that produces a 0.35 Tesla magnetic field with a useful aperture of 6.5 cm diameter, which corresponds to a hydrogen nucleus resonant frequency of 15 MHz. Each procedure produces five contiguous sections, each 5 mm thick with a spacial resolution of 1 X 1 mm FW (full width). The imager supplied four spin echo images for each of the 5 sections, using the different T1 and T2 parameter combinations given by a=28 and 56 msec and b=0.5 and 1.0 sec. These images were then used to calculate the T1, T2 and intensity images from which subsequent measurements were made. Using a track ball-driven cursor, regions of interest (ROI) were defined and a ROI program calculated the average T1, T2 and signal intensity for these regions.

In a second set of studies, gerbils were anesthetized with pentobarbital and underwent unilateral carotid artery ligation as described previously. Behavioral testing was performed as noted above and symptomatic animals were identified by their inability to resist a forced lateral pulsion with the extremities of the left side. Twelve hours after surgery, symptomatic animals were placed under 0.5% halothane anesthesia and femoral arterial and venous catheters were implanted. Animals were immobilized and then allowed to recover from anesthesia. Awake gerbils were then administered a pulse of 14C-2-deoxyglucose via the arterial cannula over 1-1/2 min. We recently have demonstrated that blood 2-deoxyglucose levels are substantially cleared from gerbil plasma within 45 min of intraarterial injection. The 14C concentration thus provided a reflection of regional cerebral glucose metabolism in the pre-treatment condition. Forty-three minutes after the initial injection, animals were given intravenous injections of 2 mg/kg naloxone. Two minutes following naloxone administration, a pulse of 3H-2-deoxyglucose was given through the arterial catheter. Animals were decapitated 45 min following the last injection and the

brains were immediately dissected and frozen in liquid freon cooled to -80°C by dry ice. The 3H concentration thus reflected cerebral glucose metabolism after drug treatment.

Blocks from the frozen brains were then sectioned with a cryostat, with a section thickness of 20 microns. The cryostat sections were rapidly dried. Each section, along with 14C and 3H brain homogenate standards, was exposed for autoradiography to two films in succession (SB5 x-ray film for low sensitivity and LKB Ultrofilm for relatively high sensitivity to 3H). Optical density was then measured for the standards and each section of interest on both films using a DeAnza IP6000. By reference to digitized densities of the standards, isotope concentrations present at each point in the section were calculated (Stryker, in preparation). The data presented indicate relative values of local cerebral glucose utilization. Knowledge of the "lumped constant" for gerbils (7), and each individual animal's plasma glucose and 2-deoxyglucose concentrations as a function of time would allow a multiplicative correction of these values to units of mg glucose/g brain per minute. For the current studies, we have assumed that animals studied by autoradiography have the same plasma glucose and 2-deoxyglucose curves as a previous group of gerbils for which these curves were measured. Regions of interest were then described and a ROI program was used to calculate both the pre- and post-treatment metabolic rates for these regions.

Results

NMR Studies

Of the 132 operated gerbils, 44 manifested demonstrable neurologic deficits, representing a stroke rate of 33%. Histologic analysis confirmed the presence of right-sided ischemic infarction in selected symptomatic gerbils. The symptomatic gerbils began to manifest signs of stroke as soon as they recovered from anesthesia, and none developed symptoms more than 4 hr after surgery. The most frequently observed neurologic deficit was hemiparesis, as manifested by a paucity of left-sided movement and a failure to resist a forced lateral pulsion with the extremities of the left side.

Asymptomatic gerbils exhibit no differences between the control and occluded hemispheres with respect to either the relative signal intensity, T1 or T2 relaxation times of the NMR image. These values remained at control levels throughout the time course of the experiment. In contrast, symptomatic animals demonstrate significant differences both between hemispheres and as compared to asymptomatic controls in both T1 and T2 times (fig. 1) as well as in three of four intensity images. The ischemic lesion could first be reliably detected as early as 3 hr after surgery, our earliest time point, in the T2 ($p<0.05$) and in the three intensity images ($p<0.001$).

T1 and T2 relaxation times appear to increase in a linear fashion over the 24 hr time course of the experiment. Increases in T2 relaxation times reach significance at the 3 hr time point and by 12 hr are highly significant ($p<0.001$). By the 24 hr time point, T2 values for the ischemic hemisphere are approximately 130% that for asymptomatic controls (70.8 ± 4.2 versus 55.2 ± 0.7; $p<0.001$). The T1 relaxation time is increased by 11% and 17% as compared to controls at the 3 hr and 12 hr time points, respectively, although these differences are not statistically significant. By the 24 hr time point, the T1 times for the occluded hemisphere rose to 147% of the control side ($1.413 \pm .090$ versus $0.961 \pm .044$; $p<0.001$).

Despite reports that naloxone reverses the neurologic deficits seen in animals following acute cerebral ischemic lesions, the results fail to demonstrate

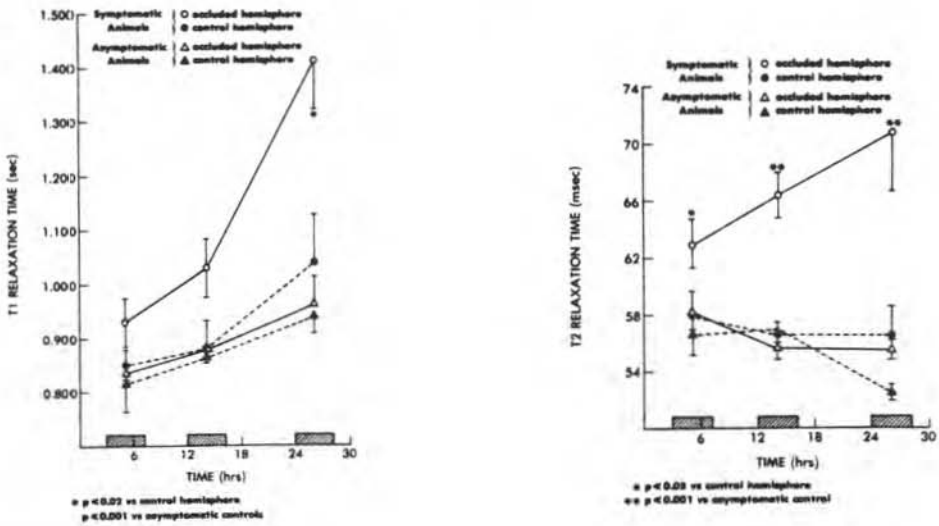

FIG. 1

T1 (left) and T2 (right) relaxation times versus time after unilateral carotid occlusion in gerbils. Darkened symbols represent the control hemisphere while empty symbols represent the occluded hemisphere.

an effect of this opiate antagonist on any of the NMR image parameters evaluated. Upon comparison of the initial NMR image to that obtained 6-10 min following naloxone administration, no significant differences between the pre-naloxone and post-naloxone conditions were observed at any time point over the 24 hr testing period. Morphine sulfate administration also had no effect on any of the parameters of the NMR image.

Metabolic Studies

The relative values for regional cerebral glucose metabolism both before and after naloxone treatment are listed in Table I. While the technique is valid for the measurement of glucose metabolism in the control hemisphere and in midline structures, the severe limitation of blood flow to the ischemic hemisphere may invalidate the assumption of the 2-deoxyglucose method that glucose uptake is in a steady state limited by metabolic demand rather than supply. The measured isotope concentrations may thus not accurately reflect cerebral metabolism in these regions. In addition, the measurement of isotope concentration in the markedly ischemic region was potentially inaccurate at the autoradiographic exposure time (optimal for the adequately perfused regions) used in this study. For these regions, we have not used the values of metabolic rate in the ischemic regions for comparison. The results indicate that while no significant changes in metabolic rate could be measured immediately about the ischemic region or laterally placed contralateral structures, there were marked focal increases in the metabolic rate in several subcortical nuclei bilaterally following naloxone injection. While no significant change was noted in the thalamus or arcuate nucleus, naloxone produced a significant elevation in the glucose metabolic rate in the substantia nigra, periaqueductal grey and the red nucleus.

TABLE I

Effect of Naloxone on Regional Glucose Metabolism
in Acute Cerebral Ischemia

Region	Control Hemisphere Pre-	Post-	%Change	Ischemic Hemisphere Pre-	Post-	%Change
Motor Cortex	10.18	10.32	+ 1	5.41	4.72	*
Sensory Cortex	10.83	11.34	+ 5	4.72	4.33	*
Caudate/Putamen	10.22	10.37	+ 1	5.34	6.21	*
Globus Pallidus	9.55	11.38	+16	2.91	4.00	*
Thalamus	9.77	10.74	+10	4.96	3.84	*
Arcuate Nucleus	11.66	12.34	+ 6	7.29	7.86	+ 5
Substantia Nigra	10.98	18.05	+65	15.95	22.47	+41
PAG	11.33	19.22	+70	10.97	18.02	+64
Red Nucleus	10.93	17.00	+56	12.15	18.26	+50

*see text

Discussion

The first series of studies indicates that cerebral ischemia following unilateral carotid artery ligation in symptomatic gerbils appears to be reflected in both T1 and T2 relaxation times, as well as in the relative hemispheric signal intensity. These changes appear to increase over the first 24 hrs after surgery. The increases in T1 and T2 times and the increasing hemispheric contrast in signal intensity most likely reflect the local edema known to develop following ischemic cerebral lesions. This hypothesis gains support from the observation by ourselves and others (8) that following carotid ligation in gerbils, the ischemic hemisphere contains significantly more water than does the control side. As T1 and especially T2 are acutely sensitive to changes in tissue water content, changes in these variables following ischemic lesions appear to reflect post-ischemic edema.

The effect of naloxone in reversing neurologic deficit secondary to cerebral ischemia has been well documented. The mechanism of this effect remains to be elucidated. We have demonstrated previously that the behavioral effect of naloxone is accompanied by a decrease in blood flow to the ischemic cortex in cats following middle cerebral artery occlusion (4). Baskin, et al. (3) have demonstrated that the effect of naloxone is not accompanied by changes in systemic arterial pressure or other cardiovascular parameters. In the current experiment, naloxone administration failed to alter the NMR image of the ischemic gerbil brain. While the lack of effect may be due to the concurrent pentobarbital anesthesia, it is also quite possible that the behavioral effect of naloxone is not due to a lessening of ischemic cerebral edema. The rapid onset and cessation of naloxone action in this system tend to support this hypothesis. In a parallel study, the NMR image of asymptomatic animals following unilateral carotid artery ligation was unaltered by the administration of morphine sulfate. Once again, it appears that the effect of opiates and opiate antagonists on the neurologic deficits secondary to cerebral ischemia are not mediated by alterations in ischemic cerebral edema.

Metabolic studies indicate that glucose metabolism is markedly diminished throughout the ischemic hemisphere. While no effect of drug treatment was noted on these ischemic regions, naloxone administration significantly increased the glucose metabolic rate in several subcortical nuclei, including the substantia nigra, periaqueductal grey and the red nucleus. Thus, while the

beneficial effects of opiate antagonists on neurologic deficits following acute ischemic cerebral lesions do not appear to be associated with a restoration of circulation or a decrease in post-ischemic edema, they are associated with a stimulation of the metabolic activity of subcortical nuclei involved with the regulation of motor activity. This effect on brainstem motor nuclei may thus allow better control of motor function in the continued absence of normally functioning cortical input.

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