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Effects of naloxone on tissue oxygen supply and somatosensory evoked potentials in cat brain during focal cerebral ischemia[1]

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ABSTRACT The effects of naloxone on local tissue oxygen partial pressure (pO_2) and on the somatosensory evoked potentials (SEP) were studied in the brain of cat during focal cerebral ischemia. Ischemia, produced by clamping of the middle cerebral artery (MCA) by a transorbital approach, was performed in two series of cats for 3 h. In one group of cats ($n=5$), naloxone 5 mg · kg^{-1} was injected iv 0.5 h after clamping. The pO_2 was continuously recorded on different depths (0 – 5000 μm) of the median gyrus by a polarographic oxygen microelectrode.

After MCA clamping, pO_2 (depth of 0 – 1000 μm) decreased markedly and hypoxia occured in the ischemic area. But in the deeper brain (1001 – 5000 μm) pO_2 did not change significantly. The amplitude of SEP decreased after MCA clamping, while the conduction time of SEP did not significantly decrease. The mean pO_2 values in the ischemic area were increased as compared to the control group after naloxone, especially at the depths of 0 – 1000 μm, and the hypoxia was improved. The amplitude of SEP was increased after naloxone in comparison to the situation of ischemia without naloxone. The conduction time of SEP was not improved significantly.

We conclude that naloxone can improve the oxygen supply and the electrical activity of neurons in the ischemic region of the brain.

KEY WORDS naloxone; oxygen; partial pressure; polarography; somatosensory evoked potentials; cerebral ischemia; cerebral arteries; cats

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Some investigators reported that opiate receptor blocker naloxone might be an effective agent for protecting ischemia[1–3], but others found no improvement in laboratory preparations[4]. As there are no data available about oxygen supply of the ischemic brain after naloxone especially the tissue oxygen partial pressure (pO_2) in deep brain tissue, we investigated the oxygen supply of the brain of the cat with naloxone induced after clamping of middle cerebral artery (MCA) by directly recording the pO_2 at different depths of brain with a new polarographic oxygen microelectrode. In some experiments the effect of naloxone on the somatosensory evoked potentials (SEP) was examined.

MATERIALS AND METHODS

Prepartion of cats Eighteen adult cats of either sex weighing 3.0 ± SD 0.4 kg were used. Cats were premedicated with im atropine 0.03 mg · kg^{-1} and ketamine 30 mg · kg^{-1}. The right femoral artery and vein were cannulated for recording the systemic arterial blood pressure and for medication, respectively. Arterial blood pressure was measured with a pressure transducer connected to a physiological recorder (STY–3000 B, China). Rectal temperature was stabilized at 38℃ by a heating pad. The cat head was placed to the prone position in a stereotactic frame with the sharpened ear bars fixed in the external ear canals.

Craniectomy about 10 mm in diameter was made over the right parietal region with a dental drill. The operative field was superfused with warm (37℃) saline. The dura mater was removed in the frontal part of the

gyrus ectosylvius medius for positioning the oxygen microelectrode. The right middle cerebral artery (MCA) was prepared by a transorbital approach[5].

Measurement of pO_2 pO_2 was measured with a platinum polarographic oxygen microelectrode[6] made from platinum wire of 0.5 μm in diameter pulled in glass with tip diameter less than 1.0 μm, which covered with a very thin layer of Ag–AgCl as coaxis reference electrode. Oxygen microelectrodes were inserted gradually from brain surface to depth of 5000 μm. The oxygen microelectrodes were calibrated immediately before and after each study period. The calibration curves were made in normal saline at 37℃ in using 99.99% N_2 and air (20.9% O_2).

Recording of SEP SEP was determined using nervous function system (Neurotrac, USA). In all experiments, SEP was recorded successively. The screw electrode was placed in the skull contralateral to the stimulating electrode and 1 cm lateral to the midline on the coronal suture and in the midline over the frontal sinus for recording the SEP. The position of this electrode was finely adjusted to obtain a typical pattern of response at the control period. Another recording electrode was placed at Erb's point for recording Erb's potentials. Amplitudes (peak to peak amplitude of the primary positive / negative response) and latencies were read to 0.1 μV and 0.1 ms. respectively. The central conduction time of the positive and negative response (CCT_p and CCT_n) were calculated.

Experimental protocol During the control period, pO_2 at different depths of puncture was recorded for 20 – 40 min. SEP was recorded once or twice as control. Group A ($n=6$), neither MCA clamping nor naloxone as nonischemic control. Group B ($n=6$), MCA was occluded for 3 h. Group C ($n=6$), naloxone (Sigma, 5 mg · kg^{-1}) was injected 0.5 h after MCA clamping. pO_2 and SEP were recorded at 0.5, 1, and 3 h after control period or MCA clamping.

Data analysis We used the unpaired t test or $F-Q$ analysis with equal variance. If the two population did not satisfy the F–test for equality of variance, the Cochran approximation was used.

RESULTS

pO_2 in the parietal brain during ischemia and nonischemia pO_2 was continuously recorded in 5 cats (group A) during the whole course of the experiments. Mean pO_2 values were quite constant at different depths of brain (Tab 1).

Tab 1. Success detection of pO_2 (kPa) at different depths of brain in nonischemia cats. $n=5$. $\bar{x} \pm$ SD. $^{*}P>0.05$, $^{**}P<0.05$, $^{***}P<0.01$ *vs* depth of 0 – 1000 μm. $^{+}P>0.05$ *vs* 0 h.

h	Depth (μm) 0–1000	1001–3000	3001–5000
0	5.0 ± 2.2	1.7 ± 0.6**	2.3 ± 2.0
0.5	4.5 ± 1.7^{+}	2.6 ± 2.3^{**+}	2.2 ± 2.1^{**+}
1.5	3.9 ± 1.3^{+}	1.5 ± 1.0^{**+}	2.0 ± 1.5^{**+}
3.0	4.5 ± 1.8^{+}	2.3 ± 1.4^{**+}	2.2 ± 0.9^{**+}

After MCA clamping, pO_2 decreased at most of the measuring points. In control group (group B), mean pO_2 values were low constant during the whole ischemic period and no significant increases in mean pO_2 values were seen at 1 and 3 h after MCA clamping (Tab 2).

Effect of naloxone on pO_2 during focal cerebral ischemia With the iv naloxone 5 mg · kg^{-1} after 0.5 h of MCA clamping, pO_2 (0 – 1000 μm) was increased as compared to the control group. The pO_2 in deeper brain was not decreased significantly after MCA clamping. With the naloxone, the increase of pO_2 values in deeper brain was insignificant (Tab 2).

Effect of naloxone on SEP during focal

Tab 2. pO_2 **(kPa) after iv naloxone 5 mg · kg^{-1} during focal cerebral ischemia in cats.** $n=5$, $\bar{x} \pm SD$, $^{*}P>0.05$, $^{**}P<0.05$, $^{***}P<0.01$ *vs* **before naloxone.** $^{+}P>0.05$, $^{++}P<0.05$ *vs* **control.**

	0–100 μm		1001–3000 μm		3001–5000 μm	
	Control	Treated	Control	Treated	Control	Treated
Preocclusion	$6.7 \pm 4.2^{**}$	$6.1 \pm 3.6^{**}$	$3.3 \pm 3.5^{*}$	$2.1 \pm 0.6^{**+}$	$3.3 \pm 3.8^{*}$	$1.5 \pm 1.6^{**+}$
Postocclusion						
Before naloxone (0.5 h)	2.3 ± 0.6	$3.0 \pm 1.4^{+}$	$2.1 \pm 1.4^{-}$	$1.7 \pm 1.2^{+}$	1.8 ± 1.7	$2.0 \pm 1.6^{+}$
0.5 h after naloxone (1 h)	$3.0 \pm 2.2^{*}$	$6.6 \pm 1.1^{***++}$	$2.0 \pm 0.9^{*}$	$3.4 \pm 2.9^{**+}$	$2.2 \pm 1.4^{*}$	$5.1 \pm 4.6^{**+}$
2.5 h after naloxone (3 h)	$3.4 \pm 3.0^{*}$	$8.2 \pm 2.3^{***++}$	2.6 ± 1.7	$2.5 \pm 1.8^{**+}$	$2.5 \pm 2.3^{*}$	$4.8 \pm 2.8^{**+}$

Note: The time of MCA clamping is given in parentheses.

Tab 3. Effect of naloxone 5 mg · kg^{-1} on somatosensory-evoked potentials during focal cerebral ischemia in cats. $n=5$, $\bar{x} \pm SD$, $^{*}P>0.05$, $^{**}P<0.05$, $^{***}P<0.01$ *vs* **control.** $^{+}P>0.05$, $^{++}P<0.05$ $^{+++}P<0.01$ *vs* **before naloxone.**

	AMP (μV)		CCT_p (ms)		CCT_n (ms)	
	Control	Treated	Control	Treated	Control	Treated
Preocclusion	$12.1 \pm 2.4^{+++}$	$13.6 \pm 3.0^{*+++}$	$7.0 \pm 1.3^{+}$	$7.0 \pm 1.1^{**+}$	$12.1 \pm 2.6^{+}$	$11.8 \pm 1.6^{**+}$
Postocclusion						
Before naloxone (0.5 h)	2.8 ± 2.5	$2.0 \pm 1.7^{*}$	6.9 ± 2.0	6.5 ± 1.6	13.3 ± 1.8	$12.2 \pm 2.4^{*}$
0.5 h after naloxone (1 h)	$2.9 \pm 2.1^{+}$	$7.0 \pm 2.3^{**+++}$	$6.5 \pm 1.4^{+}$	$9.2 \pm 3.1^{**+}$	$13.9 \pm 2.5^{+}$	$14.8 \pm 2.9^{**+}$
2.5 h after naloxone (3 h)	$1.5 \pm 1.1^{+}$	$6.6 \pm 2.1^{***++}$	$8.9 \pm 2.8^{+}$	$11.5 \pm 1.9^{*+++}$	$14.5 \pm 3.1^{+}$	$17.2 \pm 4.3^{*++}$

Note: The time of MCA clamping is given in parentheses.

cerebral ischemia The amplitude of SEP decreased significantly after MCA clamping in control and treated cats (Tab 3). In the further course of ischemia without naloxone, the amplitude of SEP did not change any more. In treated cats, the amplitude of SEP was increased 0.5 h after iv naloxone as compared to the control cats ($P<0.05$) and to pretreatment (0.5 h post-occlusion) ($P<0.05$). The CCT_p and CCT_n did not change significantly at earlier periods after MCA clamping, but in treated cat, CCT_p and CCT_n were prolonged at the later periods after iv naloxone as compared to the pretreatment, but in comparison to the control group, it was no significance.

DISCUSSION

As our results have shown that pO_2 in different measurement points (depth of 0 – 1000 μm) in the control period varied between 0 – 14 kPa (0 – 105 mm Hg) with mean pO_2 values of 5.0, 6.7, 6.1 kPa in three groups, respectively. These findings of pO_2 values are in accordance with earlier findings from cat brain and other species using surface oxygen electrodes[7,8]. In two of twelve cats clamping of the MCA produced a small but insignificant decrease in amplitude of SEP, and the recovery of pO_2 and SEP normally occured in these 2 cats within the 0.5 h after clamping. To be able to appreciate the effect of naloxone on the oxygen supply of ischemic brain area, we eliminated these two animals from our studies. In group A, one animal was also eliminated because of cerebral bleeding during puncture of oxygen microelectrode.

Recent studies have been devoted to determining whether changes in cerebral electrical activity can provide a useful clinical monitor of developing ischemia, and SEP changes

during ischemia have been related to alteration in cortical blood flow [9,10]. The amplitude reduction in SEP might be the results of reduced cortical neuronal firing[11]. In our results pO_2 in the superficial cortex and amplitudes of SEP were markedly decreased during the earlier period of MCA clamping, while the central conduction time of SEP and pO_2 in deeper brain did not change significantly. After naloxone injected the pO_2 (depth of 0 – 1000 μm) and amplitudes of SEP were significantly increased. As the electrical activity can be used as an indirect indicator for oxygen consumption of the brain cortex. We may conclude that naloxone can improve the oxygen supply and elevate the electrical activity of neurons in the ischemic area of the brain.

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纳络酮对猫局部脑缺血组织氧分压及体感诱发电位的影响

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提要 采用经眶途径结扎一侧大脑中动脉 (MCA)，造成局部脑缺血模型，用针形微型氧电极对大脑皮质缺血区不同深度 pO_2 进行连续监测. 观察到缺血后 0.5 h iv 盐酸纳络酮 5 mg · kg^{-1} 后，SEP 振幅及浅层皮质 pO_2 恢复明显优于对照组($P<0.05$, $n=5$)，而 SEP 中枢传导时间及深层 pO_2 没有明显改善. 表明纳络酮能改善大脑灰质氧的供应及增加神经细胞的电活动.

关键词 纳络酮；氧；分压；极谱法；体感诱发电位；脑缺血；脑动脉；猫