Effect of Epidural Infusion of Isobaric 2% Lidocaine on Spinal Cord Ganglionic Neurons

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We analyzed morphological and histoenzymologic changes in spinal cord ganglionic neurons of mongrel dogs caused by epidural infusion of isobaric 2% lidocaine. Lidocaine produced no pathological structural and metabolic alterations in the nervous tissue. Both epidural infusion of 0.9% NaCl and lidocaine produced some morphofunctional rearrangements in spinal ganglionic neurons. These alterations were within the limits of physiological norm and probably attested to functional response of the examined nerve tissue structures to epidural infusion.

Key Words: *lidocaine; epidural anesthesia; morphology; histoenzymology*

Local anesthetics are the major drugs for neuroaxial blockade, which are widely used in anesthesiology [2]. The appearance of new local anesthetics such as bupivacaine and ropivacaine does not moderate the wide use of domestic anesthetic lidocaine (LC), because it is produced in the forms suitable for intrathecal and epidural injections. There is evidence that spinal injections of local anesthetics affect morphologic parameters and functional activity of nerve cells and even produce a neurotoxic effect [5]. Some authors reported a local neurotoxic effect after intrathecal administration of hyperbaric 5% LC [13]. Moreover, there are data on excitatory effect of isobaric 2% lignocaine, a foreign analog to lidocaine, on spinal dorsal roots [12]. Therefore, the safety of epidural anesthesia with 2% isobaric LC is of clinical importance.

Our aim was to study the changes in canine spinal ganglionic neurons caused by chronic epidural infusion of 2% LC solution.

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MATERIALS AND METHODS

Experiments were carried out on mongrel dogs with initial body weight of 15-22 kg. The experimental dogs (n=8) received daily epidural infusions of 2% LC (Verofarm) in a dose of 5 mg/kg for 14 days. The control dogs (n=8) were daily injected with NaCl (5.0 ml 0.9% solution, physiological saline PS). Experiments were approved by Ethical Committee of I. P. Pavlov State Medical University.

After standard premedication (ketamine, 5 mg/kg 5% solution; droperidol, 0.5 mg/kg 0.25% solution; dimedrol, 1.5 mg/kg 1% solution intramuscularly) and intravenous injection of sodium thiopental (5 mg/kg), the epidural space (L_{III}-L_{VI}) was punctured with a fine (caliber 18) Twohy needle followed by catheterization with X-ray contrast catheter (caliber 20G) advanced cranially to a length of 10 cm. The distal end of the catheter was passed subcutaneously and brought out between the scapulas. The location of the catheter tip at the target level in epidural space was confirmed by X-ray examination. The test with LC (2 mg/kg 1% solution) was performed on the next day postoperation. The development of bilateral motor block during 60 sec after epidural application of LC was the criterion for inclusion of the animal in the study. On day 3

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after the end of chronic experiment, the animals were sacrificed by sodium thiopental overdose (up to 1 g).

The spinal channel was opened at Th_{II}-S_{II}, the spinal cord was exposed, and 2 segments with radices and posterior ganglia were dissected at two levels: at the catheter tip (lumbar segments, the corresponding specimens are referred to as PS^+ or LC^+) and outside the infusion zone (superthoracic segments, the corresponding specimens are marked with PS^- or LC^-). The specimens of spinal cord with radices and posterior ganglia were frozen in liquid nitrogen for 30 min after sacrifice.

For histochemical analysis of enzyme activities, cryostat sections (10 μ) were incubated with substrates by routine methods [7]. Optical density (D) of reaction products was expressed in arbitrary units. The intensity of aerobic and anaerobic processes in nerve cells was evaluated by activities of succinate dehydrogenase (SDH), cytoplasmic and mitochondrial α-glycerol phosphate dehydrogenase (GPDH), and lactate dehydrogenase (LDH). The intensity of protein synthesis was evaluated indirectly by DNA and RNA activities. The state of microcirculatory bed in nerve tissue was evaluated by alkaline phosphatase (AP) activity. The relative volume of working vessels (V_V) was counted at ×224 by the method of Weibel with a 225-point grid. Blood supply coefficient of the examined nerve tissue was calculated according to formula $K=V_V \times D$ [1]. Quantitative cytophotometry of preparations was carried out at ×200 on a single-beam cytophotometer with 1-μ optic probe diameter at the absorption maximum of 540-570 μ for the above enzymes and nucleic acids. Four visual fields, 50 cells per preparation were examined at various section levels (a total of 48 preparations).

For survey and morphometric study, the spinal cord specimens with radices and posterior ganglia were fixed in Bouin fluid, embedded in paraffin, stained with hematoxylin and eosin or Toluidine Blue by the method of Nissl. The specimens were examined in semithin (5 μ) sections, where the signs of morphological and functional alterations of the neurons were assessed at ×600 [3,10].

The data were processed statistically using Statistica SPSS 7.0 software and Student's *t* test.

RESULTS

The survey and morphometric study of specimens from control and experimental groups revealed solitary the cells with unclear borders and poorly stained Nissl substance. After epidural infusion of PS, the Nissl substance was small and granular, and it was unevenly distributed in the cytoplasm. The nuclei and nucleoli were predominantly located at the center of the neurons (Table 1). In the region of epidural LC infusion and outside it, the Nissl substance was distributed evenly throughout the cytoplasm, although the degree of granularity varied. Similar to the data obtained in PS-infused dogs, the nuclei were located predominantly at the center of the neurons, although the nucleoli were frequently shifted to nuclear periphery both in the region of LC infusion and outside it (Table 1). These morphological features indicate changes in the functional state of spinal ganglionic neurons [11]. The LC^+ and LC^- groups were characterized by eccentric location of the nucleoli in the nuclei and the presence of neurons with two nucleoli or with ectopic nucleoli shifted from the nuclei. These features attest to "depression-fatigue" effect in the neurons, which indicates changes in their functional state under the action of repeated infusions of the drug.

Activity of redox enzymes did not differ in the control and experimental groups. SDH activity was evenly distributed in both groups, which attested to stable energy metabolism in mitochondria of the nerve cells (Fig. 1). Similar data were obtained for GFDH activity, another intramitochondrial flavoprotein. The activity of cytoplasmic GPDH, which controls the transfer of electrons and H^o from extramitochondrial $NADH_2$ to mitochondrial respiratory chain [8] was comparable in neurons of all examined groups. Activity of LDH, which reflects the intensity of anaerobic metabolism [6], was somewhat higher in LC^+ group in comparison with other groups, but the differences were insignificant (Table 2).

One of the signs characterizing protein synthesis in neurons is the ratio in the numbers of nucleoli located at the center and periphery of the nucleus [9]. In this study PS^+ group nucleoli were predominantly located at the nucleus center (Table 1). By contrast, this ratio was 0.4, 0.5, and 0.6 in PS^-, LC^+, and LC^- groups, respectively. The obtained morphometric data indirectly indicate minor stimulation of protein synthesis in the neurons of PS^-, LC^+, and LC^- groups. Quantitative assessment of DNA and RNA activity revealed no significant differences between the control and experiment. The parameters obtained both in the infusion region and outside the area of drug action attested to similarity of these changes (Table 2).

Analysis of microcirculatory bed of nerve tissue revealed enhanced V_V value in PS^+ group (Table 2). The blood supply coefficients revealed no significant differences between all groups and attested to adequate state of transendothelial transporting processes in spinal ganglionic capillaries.

The results of this study support available data on high sensitivity of nerve tissue to exogenous factors related to direct transport of various therapeutic preparations to neuronal structures [4,14]. It is established

TABLE 1. Effect of Epidural Physiological Saline (FS) and Lidocaine (LC) on Morphometry of Spinal Ganglionic Cells in Chronic Experiment

Index	PS^+		PS^-		LC^+		LC^-	
	abs	%	abs	%	abs	%	abs	%
Total number of neurons	191	100	193	100	488	100	642	100
including:								
neurons with unclear borders and poorly stained Nissl substance	1	0.52	0	0	2	0.41	0	0
neurons without dystrophic alterations	190	99.48	193	100	486	99.59	642	100
Neurons with nucleus at the level of section	100	52.63	96	49.74	239	48.98	376	58.57
central nucleus	100	100	96	100	235	98.33	347	92.29
nucleus shifted to the periphery	0	0	0	0	4	1.67	29	7.71
Neurons without nucleus at the level of section	90	47.37	97	50.26	247	51.02	266	43.43
Neurons with nucleolus in nucleus	73	73.00	52	54.17	201	84.10	304	80.85
at the center	38	52.06	15	28.85	65	32.33	111	36.51
at the periphery	35	47.94	37	71.15	136	67.67	173	63.49
Neurons with ectopic nucleolus	2	2.74	2	3.85	7	3.48	4	1.32
Neurons with two-nucleolus nucleus	0	0	3	5.77	13	6.47	20	6.58
Neurons with nucleolus-free nucleus	27	27.00	44	45.83^+	34	14.22	72	23.68
Nissl granularity								
even small	0	0	23	11.92	215	44.05*	174	27.10
even large	8	4.21^+	170	88.08	273	55.94	468	72.89
uneven small	182	95.79^+	0	0	1	0.21	0	0
uneven large	0	0	0	0	0	0	0	0
none	1	0.52	0	0	1	0.21	0	0
Neurons with Nissl granularity "rim" around nucleus	2	1.05	0	0	3	1.26	10	2.66

Note. $p<0.05$: *compared to the corresponding control (PS^+ or PS^-) or $^+$compared to all other groups.

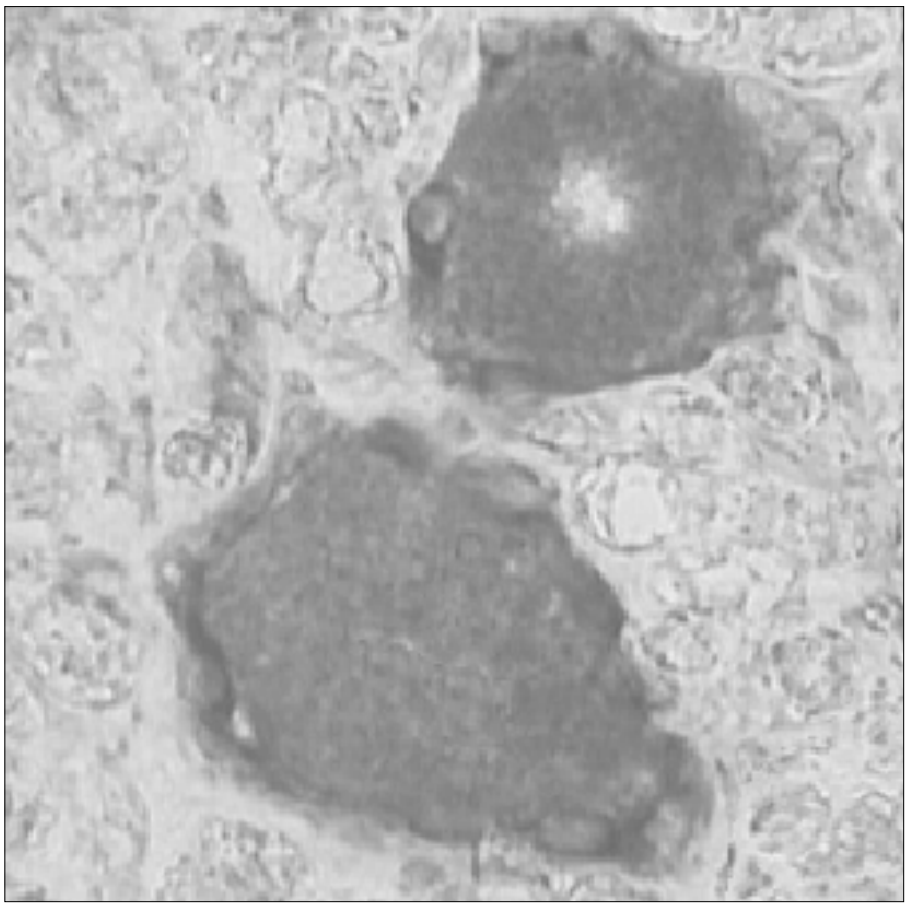

Fig. 1. Activity of succinate dehydrogenase in spinal ganglionic neurons after epidural injection of lidocaine (in the injection region). Berston staining, ×210.

that epidural LC (2%) induces morphologic and functional rearrangement in the spinal ganglionic neurons, although without the signs of neurotoxic damage. In this study we modeled LC "overload" in spinal cord structures, because duration of infusion and the single and total doses largely surpassed the corresponding clinical values. Therefore, it can be surely stated that spinal infusion of 2% isobaric LC is safe both as an individual drug for regional anesthesia and as a component of combined epidural or spinal cord analgesia.

Some pharmacokinetic and pharmacodynamic disadvantages of LC (a short-term analgesic action, rapid development of tolerance in epidural infusion) call for the search for novel and more efficient preparations for local anesthesia. At the same time, the necessity to examine their safety for both the entire organism

TABLE 2. Effect of Epidural Physiological Saline (FS) and Lidocaine (LC) on Activity of Redox Enzymes and Nucleic Acids in Spinal Ganglionic Neurons and on Microcirculatory Bed of Spinal Nerve Tissue in Dogs (Optic Density of Reaction Metabolites, Optical Density Units, *M*±*m*)

Index		PS^+	LC^+	PS^-	LC^-
SDH		0.62±0.08	0.66±0.07	0.66±0.06	0.66±0.09
GPDH	mitochondrial	0.55±0.10	0.47±0.11	0.38±0.12	0.44±0.08
	cytoplasmic	0.30±0.07	0.29±0.08	0.24±0.08	0.26±0.07
LDH		0.55±0.08	0.64±0.07	0.51±0.07	0.66±0.08
DNA		0.13±0.04	0.13±0.03	0.15±0.04	0.13±0.02
DNA+RNA		0.19±0.05	0.15±0.03	0.16±0.03	0.16±0.03
AP		0.40±0.07	0.52±0.09	0.39±0.09	0.44±0.09
Vv		8.2±0.6	6.1±0.4*	6.0±0.6*	7.2±0.4
K		3.3	3.2	2.4	3.2

Note. *$p<0.05$ compared to the PS^+ group.

and the extremely sensitive spinal cord structures at the local infusion site is evident. In this respect, LC will retain (at least in the nearest future) its leading role as a neuroaxial blocking agent, because it is an inexpensive preparation with proved safety.

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