Effect of Epidural Injection of Prosidol with Clonidine on the State of Spinal Cord and Spinal Ganglion

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Morphological and histoenzymatic changes in cells of the spinal cord and spinal ganglia after epidural injection of a combination of prosidol with clonidine were studied on dogs. No pathological structural and metabolic changes in the nervous tissue were found after combined treatment with the test drugs. Higher activity of nucleic acids and alkaline phosphatase in spinal neurons and spinal ganglion in experimental animals in comparison with those in controls indicates intensification of protein synthesis and active transport in the endothelium of nerve tissue capillaries, which is a favorable factor.

Key Words: *epidural analgesia; prosidol; clonidine; histoenzymology; morphology*

Epidural injections of opiate analgesics (morphine, fentanyl, promedol, prosidol, bupronal) and central α_2-positive agents (clonidine and guanfacine) are widely used in surgery for the treatment of pain syndromes. Opiates and α_2-agonists as epidural analgesics have undoubted advantages as well as certain limitations because of their side effects [1]. Epidural analgesia with opiates and α_2-adrenoagonists in subanalgesic doses therefore attracts special interest. This combination allows to reduce the doses of individual drugs and their main drawbacks [3]. It was shown that clonidine potentiates the analgesic activity of prosidol [6].

Clonidine injected epidurally and subarachnoidally exerted no neurotoxic effect and did not disturb blood supply to the spinal cord [7,8]. There are no publications on the effect of clonidine+prosidol combination, which hampers investigation of the possibility of clinical use of combined epidural analgesia.

We investigated the effect of epidural injection of clonidine in combination with prosidol on the morphological and histoenzymological status of the spinal cord and spinal ganglion in acute experiments on dogs.

MATERIALS AND METHODS

Experiments were carried out on 12 mongrel dogs (12-20 kg). Experimental dogs (n=5) were epidurally injected with 1% prosidol in a dose of 0.65 mg/kg+ 0.01% clonidine in a dose of 6.5 mg/kg (both from Moscow Endocrine Plant) in 0.5 ml 0.9% NaCl solution; controls (n=3) were epidurally injected with 0.9% NaCl; 4 dogs were intact.

Twenty minutes after standard premedication (droperidol, 0.5 mg/kg 25% solution, diphenhydramine, 1.5 mg/kg 1% solution; dipyrone, 50 mg/kg 50% solution intramuscularly) an intravenous catheter was inserted for fractionated infusion of sodium thiopental (4-5 mg/kg). The epidural space (L_{II}-L_V) was punctured without perforation of the dura mater with a fine Twohy needle. Normal saline and prosidol+clonidine combination was infused for 90-100 min. The animals were sacrificed by intravenous sodium thiopental overdose. The spinal channel was opened at Th_{XII}-L_{VI}, the spinal cord was exposed, and 2-3 segments with radices and posterior ganglia at the site of injection were dissected. Experiments were approved by Ethics Committee of I. P. Pavlov State Medical University.

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For morphological analysis the materials were fixed in Bouin fluid, embedded in paraffin; 5-μ sections were stained with hematoxylin and eosin and by Van Gieson and Nissl methods. The following parameters were evaluated in the ganglia: the presence and location of the nucleus and nucleoli; distribution of the Nissl substance in the cytoplasm, size of granules, and formation of a rim from Nissl granules around the nucleus.

For histochemical analysis of enzyme activities, cryostat sections (10 μ) were incubated with substrates by routine methods. 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), lactate dehydrogenase (LDH) detected by the method of Berston. The state of microcirculatory bed in nerve tissue was evaluated by alkaline phosphatase (AP) activity measured by the method of Lloyd. The intensity of protein synthesis was evaluated by DNA and RNA activities [6].

Quantitative cytophotometry of preparations was carried out at ×200 on a single-beam cytophotometer with 1-μ optic probe diameter by the plug method at different wavelengths (540-570 μ) in the maximum absorption for the above enzymes and nucleic acids. Optical density of reaction products was expressed in arbitrary units. Three-four visual fields, 50 cells per preparation were examined (a total of 48 preparations). The data were processed by the standard statistical methods.

RESULTS

Examination of spinal cord and spinal ganglion preparations by classical histological methods revealed no statistically significant differences between the experimental, control, and intact animals (Tables 1 and 2). No degenerative changes in ganglions were detected in all groups (Fig. 1). Nissl bodies was evenly distributed in the neuronal cytoplasm of the anterior and posterior horns in all groups. Most ganglionic cells had central nuclei. After epidural infusion of combination of prosidol with clonidine the nucleolus in neurons of both the spinal cord and spinal ganglion was often shifted to the periphery. In experimental group 6.35% ganglionic cell contained two nucleoli.

Activities of nucleic acids participating in the neuronal protein metabolism attested to uniform mor-

TABLE 1. Morphometry of Ganglionic Cells in Spinal Cord

Parameter	Intact (*n*=102)		Normal saline (*n*=110)		Prosidol+clonidine (*n*=73)	
	abs.	%	abs.	%	abs.	%
Neurons with unclear borders and poorly stained Nissl substance	0	0	0	0	0	0
Neurons without dystrophic changes	102	100	110	100	73	100
Neurons with nucleus at the level of section	85	83.33	102	92.72	46	63.01
including those with:						
central nucleus	71	83.55	91	89.21	39	84.78
nucleus shifted to the periphery	14	16.4	11	10.78	7	15.21
Neurons without nucleus at the level of section	17	16.67	8	7.27	51	69.86
Neurons with nucleolus in nucleus	76	89.41	95	93.1	38	82.6
including those with nucleolus:						
in the center	46	60.53	76	80	16	42.1
at the periphery	30	39.47	19	20	22	57.89
Neurons with nucleus without nucleolus	9	10.59	7	6.86	8	17.39
Nissl granules						
even small	43	42.1	22	20	1	1.36
even large	56	55	88	88	72	98.6
uneven small	0.6	0.58	0	0	0	0
uneven large	2	1.96	0	0	0	0
none	1	0.9	0	0	0	0

Note. Here and in Table 2: *n*: total number of studied neurons in the group.

TABLE 2. Morphometry of Ganglionic Cells in Spinal Gangia

Parameter	Intact (*n*=150)		Normal saline (*n*=150)		Prosidol+clonidine (*n*=173)	
	abs.	%	abs.	%	abs.	%
Neurons with unclear borders and poorly stained Nissl substance	0	0	0	0	23	13.29
Neurons without dystrophic changes	150	100	150	100	150	88.43
Neurons with nucleus at the level of section	74	49.33	81	54	92	61.33
including those with:						
central nucleus	73	98.65	78	96.29	82	89.13
nucleus shifted to the periphery	1	1.35	3	3.7	10	10.86
Neurons without nucleus at the level of section	76	50.66	69	46	58	38.66
Neurons with nucleolus in nucleus	60	81.08	44	54.32	63	68.47
including those with nucleolus:						
in the center	43	71.66	37	84.09	16	25.40
eccentric	17	28.33	7	18.9	43	68.25
Neurons with two nucleoli in the nucleus	0	0	0	0	4	6.35
Neurons with nucleus without nucleolus	14	18.91	37	45.67	29	31.52
Nissl granules						
even small	89	59	65	43	107	71.33
uneven small	0	0	12	8	1	0.66
even large	61	41	73	48.66	40	26.6
uneven large	0	0	0	0	2	1.33
Neurons with a rim of Nissl grains around the nucleus	16	10.66	2	1.33	4	2.66

phological and histoenzymological changes in protein synthesis in cells of the spinal ganglia and spinal cord in all groups (Table 3).

The functional state of neurons after epidural infusion of prosidol with clonidine was evaluated by activities of dehydrogenases (SDH, cytoplasmic and mitochondrial GPDH, LDH) participating in redox reactions of the aerobic and glycolysis cycles and characterizing the state of energy metabolism in cells [5]. No differences in activities of these enzymes in neurons of the spinal cord and spinal ganglia were detected in the three groups (Table 3), but it should be noted that optical density of dehydrogenases (except the mitochondrial GPDH) in spinal ganglia was higher than in the spinal cord.

Hydrolytic enzyme AP is the marker of vasculocellular contacts and vascular permeability [4]. AP activity of was virtually the same in the spinal ganglia

TABLE 3. Activities of Nucleic Acids and Redox Enzymes (Optical Density Units) (*M*±*s*)

Parameter	Spinal cord			Spinal ganglia		
	intact	control (normal saline)	prosidol+ clonidine	intact	control (normal saline)	prosidol+ clonidine
DNA	0.19±0.04	0.18±0.03	0.20±0.05	0.19±0.05	0.16±0.04	0.19±0.04
RNA	0.24±0.05	0.22±0.03	0.26±0.06	0.26±0.05	0.24±0.04	0.26±0.05
SDH	0.16±0.06	0.17±0.06	0.16±0.04	0.34±0.16	0.35±0.14	0.34±0.11
GPDH						
cytoplasmic	0.34±0.05	0.33±0.08	0.33±0.09	0.36±0.11	0.31±0.11	0.36±0.08
mitochondrial	0.56±0.09	0.52±0.10	0.56±0.09	0.41±0.18	0.40±0.15	0.42±0.18
LDH	0.46±0.09	0.36±0.07	0.44±0.08	0.62±0.11	0.63±0.08	0.68±0.10

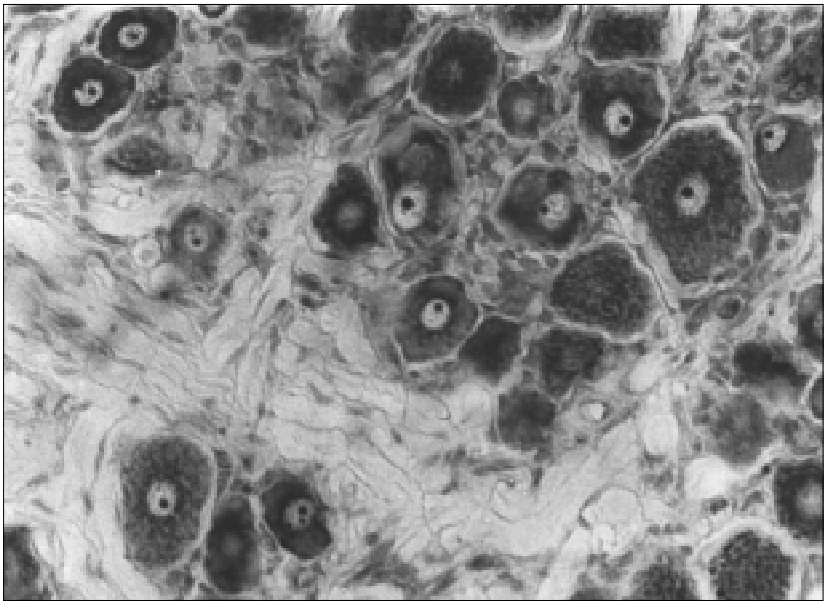

Fig. 1. Ganglionic cells of spinal ganglion after injection of prosidol with clonidine. Nissl staining, ×100. Large granules in the majority of cells, most nucleoli are eccentric.

of all studied groups (Fig. 2) and trended to increase in the gray and white matter of the spine in experimental group in comparison with intact animals and even more in comparison with the control. This indicated a certain intensification of active transport in capillary endothelium of spinal tissue in experimental dogs.

No pathological changes or complications in the cardiovascular, respiratory, or nervous systems of dogs were observed after epidural infusion of prosidol+ clonidine combination.

Pervious experimental and clinical studies showed that combined use of morphine with clonidine ensured long adequate analgesia at lower doses of the components without negative effects for the patient [2]. The present results indicate adequate functional reaction of the spinal ganglionic cells and spinal ganglion to epidural injection of prosidol with clonidine. Similar normal saline, the combination of these drugs induced certain morphofunctional and histochemical changes in enzyme activities in ganglionic cells, but these changes were within the normal range of fluctuations. A higher activity of nucleic acids and AP in the spinal and spinal ganglionar neurons of experimental dogs in comparison with control and intact animals indicates higher metabolic activity of protein synthesis in these cells and active transport in the microcirculatory bed, which is a favorable factor. A negligible number of cells with unclear borders and weakly stained Nissl substance seen in classical histological analysis, the state of neurons and activities of redox enzymes, universal changes in different structures of the nervous tissue attested to the safety of epi-

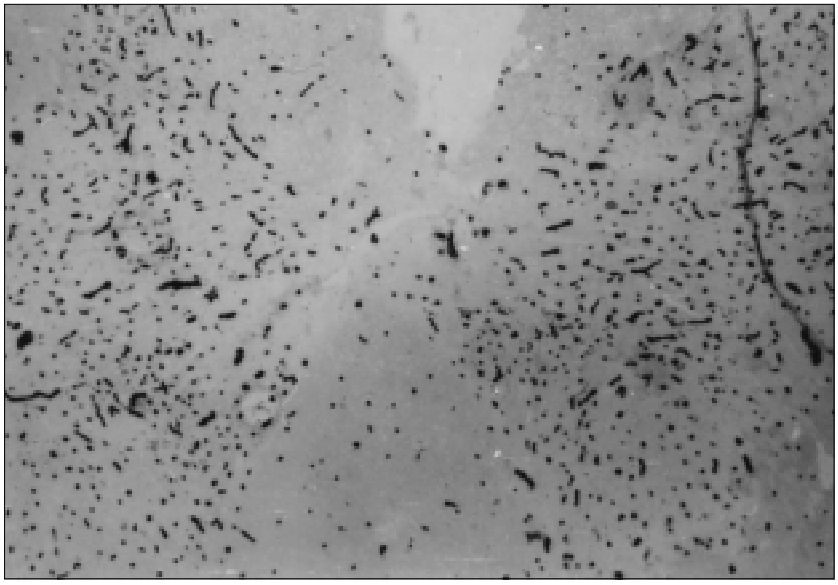

Fig. 2. Activity of alkaline phosphatase in spinal cord capillaries after infusion of prosidol with clonidine. Fast blue RR staining by the method of Berston, ×200.

dural infusion of prosidol with clonidine and prompt studies of the analgesic effect of this combination under clinical conditions for arresting painful syndromes of different origin.

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