PHARMACOLOGY AND TOXICOLOGY

Local Neurotoxicity of Epidural Clofelin

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Morphological and quantitative histoenzymological changes in neurons of dog spinal cord and spinal ganglion were studied in acute and chronic experiments with epidural administration of 0.01% clofelin. No morphofunctional changes were revealed after bolus injection of clofelin in a single dose of 6.5 mg/kg. After administration of clofelin in a daily dose of 15 μg/kg for 14 days permeability of capillaries in the nervous tissue decreased at the site of injection, but increased in intact areas. Compensatory changes in energy supply to neurons manifested in activation of aerobic and anaerobic oxidation. Sufficient level of nucleic acids synthesis confirms qualitative validity of nervous cells. Epidural clofelin did not cause dystrophy and necrosis in neurons of the spinal and spinal ganglion.

Key Words: *clofelin; neuron; epidural anesthesia*

Clofelin is extensively used in clinical practice (*e.g.*, in anesthesiology) [2,3]. Clofelin is effective in the therapy of patients with neuropathic pain and tolerance for narcotic analgesics [6]. This preparation in therapeutic doses produces an antinociceptive effect due to activation of presynaptic α_1- and α_2-adrenoceptors in the suprasegmentary and segmentary CNS, respectively [11]. Particular attention is paid to clofelin in central neuroaxial blockade, which prevents the decrease in blood flow and psychoemotional depression observed after enteral or parenteral administration of clofelin [5,8]. Selective α-adrenoceptor antagonists yohimbine and idazoxan, but not opioid antagonist naloxone, counteract the analgetic effect of spinal or epidural clofelin [12]. There are only few studies of local neurotoxicity and effect of clonidine (analogue of clofelin) on spinal blood flow [4,6,7,9,10,13]. These studies evaluated only one property of clonidine.

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We performed acute and chronic experiments to study morphofunctional changes in neurons of the spinal cord and spinal ganglia produced by epidural administration of clofelin.

MATERIALS AND METHODS

Experiments were performed on 29 outbred dogs weighing 16-20 kg. The study was performed according to ethical principles for experiments with laboratory animals. Acute experiments were conducted on 6 dogs receiving bolus epidural injection of clofelin in a single dose of 6.5 μg/kg for 100 min. In chronic experiments clofelin in a daily dose of 15 μg/kg was administered epidurally for 14 days (n=8). The control group consisted of intact animals (n=4) and dogs receiving epidurally 5.0 ml 0.9% NaCl (acute experiment, n=3; chronic experiment, n=8).

In acute experiments, samples of the spinal cord and spinal ganglia were taken from the site of injection 100 min after treatment with NaCl or clo-

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felin. In chronic experiments, 2 segments of the spinal cord and spinal ganglia were excised at 2 levels: site of location of catheter tip and injection of preparations; and intact tissue.

The methods of study, morphological and histoenzymological analysis, and statistical treatment were described elsewhere [11].

RESULTS

Acute experiments revealed no morphofunctional differences between dogs receiving bolus injection of clofelin in a single dose of 6.5 μg/kg and control animals.

Clinical signs of post-puncture lesion in the spinal cord and spinal nerves were not found in chronic experiments on dogs of both groups. A moderate sedative effect developed 10-20 min after epidural administration of clofelin. One episode of vomiting was revealed in 1 dog 15 min after clofelin injection. It was probably associated with a blood pressure drop. Visual examination after opening of the spinal channel revealed no signs of damage to the spinal cord and spinal ganglia at the site of injection and intact area. In some animals the epidural catheter was fixed to the dura mater (DM) with the connective tissue; we revealed no signs of nerve compression. Spinal ganglia were of normal appearance. Pathological changes in DM and epidural tissue were not observed during external examination. The spinal cord was positioned freely in DM. Liquor had no admixtures and looked like an opalescent yellow fluid. In spinal cord sections the boundaries between the gray matter and white matter were well defined.

Morphological study of spinal cord and spinal ganglia samples showed that most neurons have nuclei, nucleoli, and Nissl substance (Table 1). Degenerative neurons were detected in the spinal cord (site of clofelin injection, 2%; intact tissue, 0.7%) and spinal ganglion (site of clofelin injection, 6.4%; intact tissue, 1.8%). These values surpassed the corresponding parameters in control intact animals. Neurons with ectopic nucleolus were found only in chronic experiments (site of clofelin injection, 4.1%; intact tissue, 3.7%). Acute and chronic ex-

TABLE 1. Neurons in Dog Spinal Ganglia after Chronic Epidural Administration of Isotonic Sodium Chloride (SC) or Clofelin (Cl, *M*±*m*, %)

Parameter	SC, site of injection (*n*=191)	Cl, site of injection (*n*=173)	SC, intact tissue (*n*=193)	Cl, intact tissue (*n*=274)
Neurons				
dystrophic changes	3.1±0.9	6.4±1.5	0.5±0.1*	1.8±0.5*
no dystrophic changes	96.9±1.0	93.6±1.8	99.5±0.3	98.2±0.6
nucleus at the level of section	53.5±2.1	60.5±2.4	49.7±1.7	56.5±2.1
central nucleus	100.0±0.0	100.0±0.0	100.0±0.0	94.1±1.3
peripheral nucleus	0	0	0	5.9±0.9
no nucleus at the level of section	46.5±2.6	39.5±1.9	49.7±2.4	43.5±1.7
with a nucleolus in nucleus	72.7±3.1	66.3±2.3	54.2±2.6	58.7±3.2
nucleolus in the center of nucleus	52.8±3.4	16.9±2.8*	28.9±1.9*	22.6±2.3
nucleolus at the periphery of nucleus	47.2±3.3	83.1±3.7*	71.2±3.4*	77.4±3.1
ectopic nucleolus	2.80±0.23	4.1±0.3	3.9±0.3	3.7±0.4
two nucleoli in nucleus	2.8±0.2	2.0±0.2	5.8±0.7*	0.7±0.1*
nucleus without nucleoli	27.3±2.3	33.7±2.6	45.8±3.2*	41.3±3.1
Nissl substance				
regular small granules	0	0	11.9±1.2*	11.2±1.1*
regular large granules	4.3±0.9	97.5±1.3	88.1±1.5*	88.5±1.9
irregular small granules	95.7±1.2	0	0*	0
irregular large granules	0	0.6±0.1	0	0
absence	2.1±0.2	2.9±0.4	0.5±0.1*	0.7±0.1
Neurons with perinuclear granular layer	2.0±0.5	2.8±0.3	3.1±0.6	0.7±0.1*

Note. *$p<0.05$ compared to the site of injection.

TABLE 2. Oxidation-Reduction Enzymes, Nucleic Acids, in Neurons of the Spinal Cord and Spinal Ganglion and Microcirculatory Bed in Intact Dogs after Acute and Chronic Epidural Administration of Sodium Chloride (SC, arbitrary optical density units, *M±m*)

Parameter	Intact dogs		SC, acute experiment		SC, chronic experiment			
			site of injection		site of injection		intact tissue	
	spinal cord	ganglion	spinal cord	ganglion	spinal cord	ganglion	spinal cord	ganglion
SDH	0.31±0.06	0.34±0.06	0.28±0.07	0.35±0.07	0.49±0.06^{*+}	0.62±0.08^{*+}	0.58±0.08^{*+}	0.66±0.06^{*+}
GPDHmit	0.56±0.09	0.41±0.08	0.52±0.08	0.40±0.08	0.71±0.08	0.55±0.08	0.70±0.09	0.38±0.09
GPDHcyt	0.34±0.05	0.36±0.08	0.33±0.08	0.31±0.08	0.28±0.08	0.30±0.07	0.36±0.07	0.24±0.08
Lactate dehydrogenase	0.46±0.09	0.62±0.09	0.36±0.07	0.63±0.08	0.51±0.07	0.55±0.08	0.44±0.07	0.51±0.07
DNA	0.19±0.04	0.19±0.05	0.18±0.03	0.16±0.04	0.13±0.03	0.13±0.04	0.14±0.03	0.15±0.04
RNA	0.24±0.05	0.26±0.05	0.22±0.03	0.24±0.04	0.17±0.03	0.19±0.05	0.19±0.04	0.16±0.03^{*+}
Alkaline phosphatase	0.44±0.06	0.58±0.05	0.35±0.07	0.52±0.08	0.53±0.09	0.40±0.07*	0.55±0.06^{+}	0.39±0.09*
Relative volume of functioning vessels	6.6±0.4	6.8±0.5	8.4±1.1	10.0±1.0*	8.60±0.08*	8.2±0.6	5.5±0.7^{+}	6.0±0.6^{+}
Blood supply index	2.9±0.2	3.9±0.3	2.9±0.2	5.2±0.3*	4.6±0.2*	3.3±0.3	3.3±0.2^{+}	2.3±0.2^{*+}

Note. $p<0.05$: *compared to intact animals; $^{+}$compared to acute experiment. Here and in Fig. 3: GPDHmit and GPDHcyt, mitochondrial and cytoplasmic a-glycerophosphate dehydrogenase, respectively.

periments showed that spinal ganglia include cells with binucleolate nucleus at the site of clofelin injection. We revealed no morphological differences between treated and control animals.

Chronic epidural administration of clofelin was accompanied by an increase in activity of succinate dehydrogenase (SDH) in nerve cells of the spinal cord (site of injection, 0.61±0.08 arbitrary optical density units; intact tissue, 0.43±0.08 arbitrary optical density units) and spinal ganglia (0.64±0.07 and 0.59±0.08 arbitrary optical density units, respectively). SDH activity in these dogs significantly differed from enzyme activity in intact animals and dogs with acute administration of clofelin. Activity of mitochondrial α-glycerophosphate dehydrogenase in neurons of the spinal cord increased significantly at the site of clofelin injection (0.85±0.08 arbitrary optical density units, $p<0.05$ compared to the control). Activities of cytoplasmic α-glycerophosphate dehydrogenase and lactate dehydrogenase in treated dogs did not differ from those in control animals and dogs with acute administration of clofelin. Activities of neuronal DNA and RNA slightly differed between animals receiving acute and chronic injections of clofelin (Tables 2 and 3).

Chronic epidural administration of clofelin had a potent effect on the microcirculatory bed in nervous structures. Alkaline phosphatase activity in neurons of the gray matter (0.79±0.04 arbitrary optical density units) and white matter (0.74±0.07 arbitrary optical density units) in intact spinal cord of treated dogs was higher compared to other animals ($p<0.05$). Alkaline phosphatase activity was lowest in the gray matter, white matter, and spinal ganglion at the site of clofelin injection (0.38±0.06, 0.35±0.08, and 0.21±0.05 arbitrary optical density units, $p<0.05$). Other parameters of microcirculatory function in the nervous tissue (relative volume of functioning vessels and coefficient of blood supply) underwent similar changes (Tables 2 and 3).

Previous studies showed that spinal or epidural administration of clofelin in doses of 3-10 μg/kg is not followed by reduction of blood flow in the spinal cord and nerve roots or development of damage to the nervous tissue [4,6,7,9]. There are ambiguous data on the effect of long-term treatment with clofelin in high doses on spinal blood flow and morphological characteristics of the spinal cord. For example, acute administration of clonidine in increasing doses (up to 750 μg) into the spinal cord produced no adverse effects on blood supply to the kidneys, spinal cord, and nerve roots in sheep and dogs [6,9]. Experiments on dogs revealed no signs of local neurotoxicity after epidural administration of clonidine in a dose of 320 μg/kg (daily dose 7.7

TABLE 3. Oxidation-Reduction Enzymes, Nucleic Acids, and Microcirculatory Bed in Dogs after Acute and Chronic Epidural Administration of Clofelin (arbitrary optical density units, *M*±*m*)

Parameter	Acute experiment		Chronic experiment			
	site of injection		site of injection		intact tissue	
	spinal cord	ganglion	spinal cord	ganglion	spinal cord	ganglion
SDH	0.30±0.05	0.33±0.09	0.61±0.08*	0.64±0.07*	0.43±0.08	0.59±0.08*
GPDHmit	0.56±0.09	0.42±0.09	0.85±0.08 *	0.48±0.08	0.73±0.07	0.35±0.08
GPDHcyt	0.33±0.09	0.30±0.08	0.31±0.08	0.26±0.07	0.29±0.07	0.29±0.08
Lactate dehydrogenase	0.40±0.07	0.67±0.10	0.57±0.09	0.62±0.09	0.41±0.09	0.43±0.07
DNA	0.19±0.04	0.16±0.04	0.12±0.04	0.12±0.03	0.13±0.03	0.14±0.02
RNA	0.25±0.05	0.24±0.04	0.20±0.04	0.19±0.04	0.21±0.03	0.20±0.04
Alkaline phosphatase	0.56±0.08	0.55±0.08	0.37±0.06*	0.21±0.05*	0.77±0.05*	0.49±0.10
Relative volume of functioning vessels	7.8±0.5	8.2±0.8	4.7±0.7	6.1±0.8	7.4±0.8	7.7±0.5
Blood supply index	4.4±0.3	4.5±0.4	1.8±0.2*	1.3±0.1*	5.7±0.4*	3.8±0.3

Note. $p<0.05$: *compared to acute experiment.

mg) for 12-28 days. However, these authors found morphological changes in the nervous tissue near the catheter tip location (reactive inflammation, infiltration with lymphocytes and polymorphonuclear leukocytes, and increased vacuolization of cells) [13].

Experiments on rats, dogs, and pigs showed that administration of 10-30 μg/kg clonidine into the spinal cord for 14 days decreased spinal blood flow by 20-39% compared to the control [4,7]. Most neurons at the site of injection retained normal morphological characteristics, while some nerve cells were characterized by various forms of degeneration.

Our results are consistent with published data on morphological changes and variations in local blood flow produced by epidural administration of clofelin [4,7]. Injection of clofelin in a single dose of 6.5 μg/kg produced no pathological morphological and histoenzymological changes in neurons of the spinal cord and spinal ganglion. Epidural administration of clofelin in a 10-fold therapeutic dose for 14 days decreased microcirculatory function at the site of injection (by 36-55%) and impaired capillary—neuronal interrelations. Treated animals had a greater number of structurally modified neurons (compared to the control). Examination of tissues distant from the site of clofelin injection showed that parameters of blood flow in the spinal cord and spinal ganglion of treated dogs slightly exceeded those in control animals. The increase in the function of microcirculatory bed in areas distant from the site of clofelin injection was probably associated with progressive decrease in the concentration of epidural preparation with its cranial distribution.

Our results indicate that epidural administration of clofelin does not induce degeneration and necrosis. Therefore, acute and chronic epidural administration of clofelin in doses of 3-10 μg/kg is clinically safe. Chronic epidural administration of clofelin in a higher dose (10 μg) increases the risk of ischemic and necrobiotic damage to the spinal cord.

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