Effects of Leu-Enkephalin and Dalargin on Cardiac Function in Rats under Conditions of Low-Frequency Stimulation of Postganglionic Sympathetic Fibers

F. G. Sitdikov and T. G. Makarenko

Translated from *Byulleten' Eksperimental'noi Biologii i Meditsiny*, Vol. 127, No. 2, pp. 140-143, February, 1999
Original article submitted March 24, 1998

Leu-enkephalin inhibits and dalargin has no significant effect on the positive chronotropic effect of sympathetic stimulation. Both peptides potentiate the positive inotropic effect but do not change cardiac output.

Key Words: *postganglionic sympathetic fibers; leu-enkephalin; dalargin; low-frequency stimulation; cardiac action*

High activity of opioid peptides in the regulation of cardiac function is generally known. The opioidergic modulation of the cardiac control can be realized at various levels of the autonomic nervous system [4]. Opioid peptides inhibit chronotropic sympathetic effect [1,2,6,8,9]. However, their influence on inotropic sympathetic effect, i.e. on stroke volume (SV) and cardiac output (CO) remains virtually unknown. The synthetic leu-enkephalin analog dalargin is more stable in biological media and produces more sustained effect than the original peptide. However, the differences in chemical structure determine different physiological properties of these peptides.

Our aim was a complex study of the effects of these opioid peptides on sympathetic regulation of the cardiac function. Specifically, we examined the effects of leu-enkephalin and dalargin on heart rate (HR), SV, and CO during low-frequency stimulation of the postganglionic sympathetic fibers in rats.

MATERIALS AND METHODS

The study was carried out on 25 mature albino rats (body weight 130-160 g) anesthetized with urethane (1.3 mg/kg intraperitoneally) and artificially ventilated. The right stellate ganglion was cleaned from the adjacent tissues, slightly lifted, and placed on platinum electrodes. Benzohexonium (10 mg/kg) was injected into the left jugular vein and 5-10 min later stimulation of the sympathetic ganglion was started (0.5, 1, 2, and 4 Hz frequency, 5 msec pulse duration, and 10 V pulse amplitude). Duration of the stimulation was 30 sec, and rheogram was recorded during 5 sec. HR, SV, and CA were calculated from the rheogram recorded using a RPG-4 rheograph and one-channel ink recorder. Needle electrodes were fixed subcutaneously on the head and hind limb (current electrodes) and on the ventral surface of the body on the line going along the clavicles, and also on the dorsal surface of the thorax along the arch going between the median axilar lines (measuring electrodes). The nerve fibers were stimulated with an ESL-2 electrical stimulator. After recording of the rheogram, the stimulation series was repeated against the background of leu-enkephalin (56.7 mg/kg). The drug was injected before each stimulation because of short halflife (2 min). The same stimulation series was then performed against the background of dalargin (100 mg/kg). The data were analyzed statistically using Student's t test at $p<0.05$. Leu-enkephalin and dalargin were synthesized at the Vector Research-and-Production Association (Kol'tsovo, Novosibirsk region).

RESULTS

Stimulation of the postganglionic sympathetic fibers increased HR in a frequency-dependent manner (Table

Department of Human Anatomy, Physiology, and Health Protection, Kazan Pedagogical University

0007-4888/99/0002-0126$22.00

1). Stimulation against the background of leu-enkephalin injection produced a less pronounced positive chronotropic effect at 0.5, 2, and 4 Hz ($p<0.05$), but not at 1 Hz ($p>0.05$). Dalargin also inhibited the positive chronotropic effect, but these changes were insignificant (Table 1).

Stroke volume in mature rats was 0.14±0.02 ml. Stimulation at a frequency of 0.5 Hz insignificantly increased SV (Table 2), while stimulation at 1, 2, and 4 Hz decreased this parameter. Hence, stimulation of postganglionic sympathetic fibers in rats produces insignificant changes in SV. Leu-enkephalin potentiated the positive chronotropic effect at 1 and 4 Hz (Table 2), while dalargin little affected SV.

Neither stimulation, nor opioid peptides significantly affected CO in mature rats (Table 3).

Our findings showed that opioid peptides inhibit the sympathetic cardiochronotropic effects in mature rats. Presumably, leu-enkephalin acts on the presynaptic membrane and inhibits the release of norepinephrine from sympathetic terminals (similar effects of opioid peptides have been described previously [7-9]). Since opioid peptides prevent the increase in HR and abolish or even reverse the negative effect of stimula-

TABLE 1. Effect of Leu-Enkephalin and Dalargin on HR (beats/min) in Mature Rats under Conditions of Postganglionic Sympathetic Fiber Stimulation ($M±m$)

Series	Stimulation frequency, Hz			
	0.5	1	2	4
Control				
before stimulation	330.10±15.48	344.60±14.64	339.30±15.88	338.00±16.22
after stimulation	372.30±17.74	399.14±16.54	415.87±19.70	431.00±14.00
changes, %	11.78	13.40	18.40	20.13
Leu-enkephalin				
before stimulation	328.46±19.16	339.72±15.86	335.30±16.12	343.40±16.26
after stimulation	360.60±14.72	378.18±15.52	349.41±16.90	421.50±19.65
changes, %	6.80*	9.56	12.48*	15.99*
Dalargin				
before stimulation	296.00±14.50	307.75±13.42	313.50±12.91	319.28±16.27
after stimulation	337.75±11.49	345.37±11.07	411.60±16.16	394.28±10.01
changes, %	12.80	11.52	15.06	19.36

Note. *$p<0.05$ compared to the control.

TABLE 2. Effect of Leu-Enkephalin and Dalargin on SV (ml) in Mature Rats under Conditions of Postganglionic Sympathetic Fiber Stimulation ($M±m$)

Series	Stimulation frequency, Hz			
	0.5	1	2	4
Control				
before stimulation	0.14±0.020	0.10±0.029	0.14±0.030	0.16±0.020
after stimulation	0.20±0.030	0.08±0.032	0.10±0.025	0.12±0.018
changes, %	30.00	-20.00	-28.60	-25.00
Leu-enkephalin				
before stimulation	0.16±0.020	0.12±0.025	0.14±0.030	0.11±0.020
after stimulation	0.16±0.020	0.23±0.030	0.14±0.030	0.20±0.022
changes, %	0	47.80	0	45.00
Dalargin				
before stimulation	0.16±0.020	0.15±0.019	0.15±0.028	0.14±0.026
after stimulation	0.14±0.031	0.16±0.018	0.15±0.030	0.16±0.020
changes, %	-12.00	6.00	0	12.50

TABLE 3. Effect of Leu-Enkephalin and Dalargin on CO (ml/min) in Mature Rats under Conditions of Postganglionic Sympathetic Fiber Stimulation (*M±m*)

Series	Stimulation frequency, Hz			
	0.5	1	2	4
Control				
before stimulation	41.42±3.72	30.82±4.52	41.86±3.84	40.00±2.11
after stimulation	37.39±2.00	30.07±6.25	39.75±7.34	44.23±5.63
changes, %	-9.70	-2.40	-5.00	9.60
Leu-enkephalin				
before stimulation	51.16±3.00	43.66±3.77	46.16±3.59	39.80±4.65
after stimulation	52.30±4.25	45.86±2.50	50.59±3.00	41.58±2.84
changes, %	2.18	4.80	8.80	4.30
Dalargin				
before stimulation	36.86±2.15	45.14±5.26	47.98±2.15	50.88±3.54
after stimulation	42.01±3.71	52.53±4.73	49.52±3.47	53.40±5.26
changes, %	5.10	14.10	-3.10	4.70

tion on SV, CO remains practically unchanged. The decrease in SV after stimulation was accompanied by shortening of the ejection time and by flattening of the rheographic wave, reflecting the degree of ventricular diastolic filling. The decrease in SV can be transient (we recorded the cardiac indices for only 5 sec poststimulation). Previously we showed that the force of heart contractions could decrease during the first 15 sec after stimulation, while HR in this period significantly increases [2]. Heart work depends on the initial length of myocardial fibers. The efficiency of cardiac contractions increases with increasing of the initial fiber length only to an optimal value [5]. In our experiments, stimulation of the postganglionic sympathetic fibers drastically shortened the ejection time, which resulted in incomplete myocardial contraction and explains the absence of rheographic changes.

Thus, stimulation significantly increased HR, but had no effect on CO.

REFERENCES

1. S. A. Muranevich, *Neuropeptides as Regulators of Sympathetic Enzyme Activity* [in Russian], Leningrad (1988).
2. F. G. Sitdikov, *The Mechanisms and Age-Related Features of Cardiac Adaptation to Long-Term Sympathetic Stimulation*, Author's Synopsis of Doct. Biol. Sci. Dissertation [in Russian], Kazan (1974).
3. N. A. Sokolova, *Biol. Nauki.*, No. 10, 13-24 (1987).
4. N. A. Sokolova, *Pat. Fiziol.*, No. 6, 74-79 (1988).
5. N. Sperelakis, in: *Physiology and Pathophysiology of the Heart*, Boston (1984).
6. M. Bachoo, I. Cirello, and C. Polosa, *Brain. Res.*, **400**, No. 2, 277-282 (1987).
7. F. I. Dies-Guerra, S. Augood, P. C. Emson, and R. G. Dyer, *Exp. Brain Res.*, **66**, No. 2, 378-384 (1987).
8. H. Kondo and H. Kuramoto, *Brain Res.*, **335**, No. 2, 309-314 (1985).
9. S. Konishi and A. Tsunoo, *Biomed. Res.*, No. 1, 528-536 (1980).