ANTINOCICEPTIVE PROPERTIES OF DALARGIN USED IN ANESTHESIOLOGIC PROTECTION

B. M. Shloznikov, S. G. Donich, O. A. Grebenchikov, R. F. Makhmutov, V. V. Likhvantsev, A. Yu. Kuznetsov, A. I. Peretrukhin, and E. P. Fomchenkov

UDC 615.31:[547.95:547.943].017:615.211].07

KEY WORDS: dalargin; myoplegia; hemodynamics; antinociception

Dalargin, a synthetic analog of natural Leu-enkephalin, is a regulatory opioid peptide capable of interacting with high affinity with delta-receptors, and possessing rather lower affinity for mu-opiate receptors [9]. In therapeutic doses dalargin does not pass through the blood-brain barrier (BBB), a fact which characterizes it as a peptide with peripheral action, possessing weaker analgesic properties than the stable enkephalin analog FK 33-824 [1, 2, 9].

The possibility of using dalargin during combined general anesthesia as a drug dispensing with the need to use narcotic analgesics in order to guarantee adequate anesthesiologic protection of the patient has been demonstrated in the Department of Anesthesiology and Resuscitation of the A. V. Vishnevskii Institute of Surgery, Academy of Medical Sciences of the USSR. This method differs from existing methods of general anesthesia by its freedom from the side effects of classical opioids [3, 6, 7].

The aim of this investigation was an experimental study of the antinociceptive properties of dalargin in concentrations equivalent to doses used in general anesthesia.

EXPERIMENTAL METHOD

Experiments were carried out on 131 male Wistar rats weighing 250-300 g. Under pentobarbital anesthesia (40 mg/kg, intraperitoneally), in conjunction with local anesthesia, tracheostomy was performed on the animals, after which the lungs were artificially ventilated mechanically under moderate hyperventilation conditions: tidal volume (TV) 2-2.5 ml, respiration rate (RR) 68 min^{-1}, p_aCO_2 32-34 mm Hg. To inject the preparations the right jugular vein was cannulated with PE-50 catheters. Cardiac output was determined with a "Nihon Kohden" electromagnetic flowmeter (Japan), the transducer of which was fixed to the ascending aorta. To measure the blood pressure (BP) the abdominal aorta was catheterized with a "Viggo" arterial cannula (France). Curves were traced on a Soviet "Salyut" polygraph. The following parameters were recorded and calculated: heart rate (HR), systolic (BP_s), diastolic (BP_d), and mean BP (BP_m), cardiac output (CO), cardiac index (CI), stroke volume (SV) of the left ventricle, stroke index (SI), power of the left ventricle (M_{lv}), total peripheral resistance (TPR), elasticity of the arterial reservoir (EAR), and double product (HRP). All calculations involved in these parameters were done on an "Élektronika" microcomputer, using the "Hemo" program developed jointly with E. N. Timin. Hemodynamic parameters were studied at the following stages: I) initial state, II) 20 sec after injection of the preparation, III) 10 sec after the beginning of application of the nociceptive stimulus, and IV) 60 sec after the beginning of stimulation. The animals for study were divided into four groups: 1) 14 rats subjected to total myoplegia with arduan (0.1 mg/kg) then receiving an injection of dalargin (20 μg/kg), 2) 11 rats receiving the same doses of the muscle relaxant followed by physiological saline, 3) seven rats without myoplegia and receiving physiological saline, and 4) seven rats without myoplegia receiving an injection of dalargin in a dose of 20 μg/kg. Abolition of

Department of Anesthesiology and Resuscitation, A. V. Vishnevskii Institute of Surgery, Academy of Medical Sciences of the USSR, Moscow. (Presented by Academician of the Academy of Medical Sciences of the USSR D. S. Sarkisov.) Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 109, No. 3, pp. 272-275, March, 1990. Original article submitted July 25, 1989.

 0007-4888/90/0003-0350$12.50

TABLE 1. Changes in Some Parameters of Central and Peripheral Hemodynamics in Rats with Total Myoplegia after Injection of Dalargin (D) and Physiological Saline (S), Followed by Nociceptive Stimulation ($M \pm m$)

Parameter	Preparation	Stages of investigation			
		I	II	III	IV
BP_m, mm Hg	D	70,4±3,6	63,0±2,6*	71,3±3,4	71,1±3,2
	S	64,8±3,2	65,6±3,1	80,1±4,1*	78,0±3,2*
HR, min^{-1}	D	362,8±5,2	349,9±5,0*	354,4±5,4*	353,7±5,3*
	S	356,3±2,9	356,3±2,9	371,0±3,5*	382,6±2,4*
CI, liter·min^{-1}·m^2	D	4,64±0,23	4,60±0,23	4,66±0,24	4,54±0,24#
	S	5,06±0,22	5,09±0,21	5,44±0,25*	5,78±0,27*
SI, ml·m^{-2}	D	12,81±0,63	13,19±0,66*	13,19±0,67*	12,88±0,68
	S	14,23±0,65	14,30±0,64	14,68±0,68	15,13±0,74*
M_{lv}, W	D	2,39±0,19	2,63±0,21#	2,37±0,20	2,31±0,19
	S	2,75±0,23	2,81±0,23	3,13±0,20#	3,31±0,28#
HRP, conventional units	D	309,1±11,4	278,3±9,2*	304,8±11,3	303,4±10,6
	S	286,3±12,6	290,4±12,3	359,1±16,1*	361,6±14,3*

Legend. Here and in Table 2, asterisk indicates significant differences compared with corresponding parameter in stage I (level of significance of differences $p < 0.001$); #) denotes the same, at the $p < 0.05$ level.

the hemodynamic response evoked by the nociceptive stimulus by the action of various doses of the opioid peptide (from 0.9 to 3.570 μg/kg) was studied on 72 rats, divided into 10 groups (6-8 animals in each group) under conditions of total myoplenia. The dose of dalarginreturning the hemodynamic test parameters to their original level was taken to be effective. In control experiments intravenous injection of fentanyl in a dose of 20 μg/kg completely abolished the evoked hemodynamic response.

In the next stage of the experiment the preventive and suppressive effect of the universal opioid antagonist naloxone on the normalizing action of dalargin was studied in two groups (seven and 10 rats respectively). All the investigations began with the transition from narcotic sleep to a more superficial level. The opioid peptide was injected intravenously in a volume of 0.1 ml. Nociceptive stimulation was induced by mechanical compression of the base of the tail [8] and was graded with respect to the time and force of compression. The results were subjected to statistical analysis by the method of paired comparisons, using Student's t test.

EXPERIMENTAL RESULTS

In the animals of group 1 the following changes in the parameters were found after intravenous injection of dalargin (Table 1): lowering of BP_s by 6.7% ($p < 0.001$), of BP_d by 13.1% ($p < 0.001$), of BP_m by 10.5% ($p < 0.001$), of HR by 3.6% ($p < 0.001$), of TPR by 10.6% ($p < 0.001$), and of HRP by 10% the absence of a negative inotropic action of dalargin to be indirectly judged. After application of the nociceptive stimulus, a transient rise of BP, CI, and TPR was observed, although not statistically significant, and HR remained virtually unchanged, while SI remained at its former level in stage II.

In the final stage of the investigation a significant fall of CI was observed compared with its value in stage I, by 2.2% ($p < 0.001$), and could be explained by the return of SI to its original level while HR was lowered by 2.6% ($p < 0.001$); the remaining hemodynamic parameters had virtually their initial values. The results indicate the stabilizing action of dalargin combined with myoplegia on the circulation under conditions of nociceptive stimulation.

In the animals of group 2 (Table 1) injection of physiological saline caused no significant changes in the parameters studied. The nociceptive stimulus evoked a significant increase in all hemodynamic parameters. In stage III the response of the vascular bed was most informative: BP_m rose by 23.5% ($p < 0.001$) and TPR by 17.1% ($p < 0.001$), whereas EAR fell by 12.8% ($p < 0.001$). HR, CI, SI, M_{lv}, and HRP increased progressively and reached a maximum 60 sec after 7.3, 14.1, 6.3, 20.3, 26.2% respectively (in all cases $p < 0.001$). The hemodynamic response described above indicates absence of adequate protection against nociception by either the myoplegia of the placebo.

In the animals of group 3 (Table 2) the character of the hemodynamic changes agreed with that in the previous series, but some showed a higher percentage increase. In the rats of group 4 (Table 2) intravenous injection of dalargin caused hemodynamic changes similar to those in group 1: BP_s fell by 11.4% ($p < 0.001$), BP_d by 13.3% ($p < 0.001$), BP_m by 12.5% ($p < 0.05$), HR by 3% ($p < 0.001$), TPR by 11.1% ($p > 0.05$), and HRP by 14% ($p < 0.001$). In stage III of the investigation in this group BP rose by 16.2% ($p < 0.01$) but there were no significant changes in TPR and EAR; HR, CI, SI, M_{lv}, and HRP increased steadily to reach a maximum in the last stage, with peak values of 5.3, 14.8, 9, 25.9, and 15% respectively of the original level (in all cases $p < 0.01$). These results are evidence of the inadequacy of the protective action of dalargin without myoplegia on the

TABLE 2. Changes in Some Parameters of Central and Peripheral Hemodynamics in Rats without Myoplegia and Receiving Injections of Dalargin (D) and Physiological Saline (S) Followed by Application of Nociceptive Stimulus ($M \pm m$)

Parameter	Preparation	Stages of investigation			
		I	II	III	IV
BP_m, mm Hg	D	66,7±2,2	58,4±3,1*	77,6±3,3*	72,3±3,2*
	S	63,2±3,2	63,2±3,2	79,9±2,3*	70,8±2,0*
HR, min^{-1}	D	368,0±5,5	357,2±7,8*	375,8±7,7*	387,5±6,6*
	S	364,5±8,3	364,5±8,3	379,2±8,4*	389,0±7,8*
CI, liters·min^{-1}·m^2	D	4,20±0,28	4,07±0,30	4,56±0,30*	4,82±0,33*
	S	4,22±0,20	4,22±0,20	4,70±0,19*	4,81±0,17*
SI, ml·m^{-1}	D	11,41±0,74	11,37±0,71	12,12±0,70*	12,44±0,81*
	S	11,60±0,16	11,60±0,16	12,41±0,26*	12,38±0,24*
M_{lv}, W	D	2,20±0,26	2,01±0,26	2,74±0,24*	2,77±0,14*
	S	1,97±0,13	1,97±0,13	2,43±0,25*	2,76±0,30*
HRP, conventional units	D	300,4±5,5	258,6±11,9*	356,5±10,9*	345,5±10,0*
		280,3±14,3	280,3±14,3	360,6±15,1*	340,5±10,6*

hemodynamics on the appearance of nociception. The effect of dalargin was confined to stabilization of the peripheral component of the systemic circulation.

The study of the normalizing action of dalargin on the hyperdynamic response of the circulation evoked by the nociceptive stimulus revealed a nonlinear dependence of effect on dose. Starting with a dose of 17.2 μg/kg the maximal suppressive effect of dalargin on the parameters of the central and peripheral hemodynamics chosen for study was observed and a further increase in the dose of opioid peptide to 3570 μg/kg did not lead to any significantly improved result. Naloxone, in a dose of 100 μg/kg, injected 6 min before the peptide, completely prevented the protective action of dalargin on the systemic hemodynamics in response to the nociceptive stimulus. The suppressive effect of dalargin also was abolished by the above-mentioned dose of the antagonist, injected 15 sec after dalargin.

Injection of dalargin against a background of total myoplegia and artificial ventilation of the lungs thus prevents the hemodynamic response to the nociceptive stimulus in rats. The antinociceptive properties of dalargin are well defined in a dose of 17.2 μg/kg in conjunction with antidepolarizing muscle relaxants. A further increase in the dose does not change the efficacy of the peptide. Furthermore, replacement of the narcotic analgesic by dalargin in the schedule of general anesthesia may provide adequate (relative to hemodynamic parameters) protection against surgical trauma.

LITERATURE CITED

1. E. I. Kalenikova, O. F. Dmitrieva, N. V. Korobov, et al., Neuropeptides in Experimental and Clinical Practice [in Russian], Moscow (1986), pp. 80-85.
2. N. V. Korobov, Farmakol. Toksikol., No. 4, 35 (1988).
3. A. Yu. Kuznetsov, Modern Requirements in Reconstructive Surgery [in Russian], Moscow (1988).
4. E. A. Martynova and O. S. Medvedev, Byull. Éksp. Biol. Med., No. 1, 60 (1986).
5. E. A. Martynova and O. S. Medvedev, Byull. Éksp. Biol. Med., No. 8, 136 (1988).
6. A. I. Peretrukhin, Current Problems in Reconstructive Surgery [in Russian], Moscow (1988).
7. A. V. Chichenkov, Current Problems in Reconstructive Surgery [in Russian], Moscow (1988).
8. O. N. Chichenkov and N. V. Korobov, Pharmacol. Toksikol., No. 1, 23 (1984).
9. K. H. Yarygin, Author's Abstract of Dissertation for the Degree of Doctor of Sciences, Moscow (1989).
10. R. N. Willette and H. N. Sapru, Eur. J. Pharmacol., **78**, 61 (1982).