Effect of Intravenous Injection of DAGO on Neuronal Activity in Bulbar Cardiovascular Center

S. D. Mikhailova, A. V. Sokolov, T. M. Semushkina, and G. I. Storozhakov

Translated from *Byulleten' Eksperimental'noi Biologii I Meditsiny*, Vol. 131, No. 7, pp. 48-51, July 2001
Original article submitted April 19, 2001

Effects of intravenous injection of DAGO on impulse neuronal activity of the bulbar cardiovascular center were studied in acute experiments on cats narcotized with Nembutal. DAGO-induced changes in activity of sensory neurons and interneurons were unidirectional and preceded hemodynamic shifts. The antiarrhythmic effect of DAGO is due to its direct action on neurons of the bulbar cardiovascular center.

Key Words: *opiate receptors*; *DAGO*; *myocardial ischemia*; *cardiac arrhythmias*; *bulbar cardiovascular center*

Recent studies demonstrated an important role of opioid peptides in heart protection from ischemia-induced arrhythmias. In these studies direct effects of opioid peptides on myocardial receptors [7] or central effects of opioid peptides injected into brain ventricles [6,8] were evaluated. Intravenous injections of opioid peptides DAGO and DALD produce a chronotropic effect on the heart via a neurogenic mechanism; the antiarrhythmic effect of DAGO depends on the state of cardiac innervation [4,5].

Our aim was to study the effect of a highly selective synthetic μ-opiate receptor antagonist DAGO on impulse activity of bulbar cardiovascular neurons.

MATERIALS AND METHODS

Experiments were performed on 18 artificially ventilated cats, narcotized with Nembutal (40 mg/kg, intraperitoneally). Electrical activity of bulbar cardiovascular neurons was recorded extracellularly using a Pyrex glass microelectrodes filled with 2.5 M KCl (2-4 μ tip size, 7-10 MΩ resistance).

Electrical signals were amplified with a specially designed amplifier for microelectrode recording (Physiological Department of Russian State Medical University).

The microelectrode was introduced into the brain using a Medikor stereotaxis apparatus equipped with a precision mechanical micromanipulator. The search was restricted to a region of the nucleus tractus solitarius from 2 mm rostral to 2 mm caudal to *obex* [1].

To this end, the cat was placed in a stereotaxis, occipital muscles were cut along the middle line from the occipital crest to C_{II} and then laterally, pulled apart, and fixed with ligatures. The occipital bone was cut from the base upward, and a wax was applied to prevent bleeding. The dura mater was cut along the middle line and laterally, and pulled apart with ligatures. The brain surface was treated with mineral oil to prevent drying. The manipulator with a microelectrode was set and oriented with respect to *obex*, and spikes of bulbar neurons were recorded.

After attaining stable signal, a synthetic opioid peptide DAGO (D-Ala2, ME-Phe4, Gly-ol5)-enkephalin (Peptidnyi Sintez) was infused intravenously in a dose of 20 μg/ml for 15 min.

Blood pressure (BP) in the femoral artery (EMT-35), ECG (M-42 myograph amplifier), and volume rate of respiration were continuously recorded (EMT-32C).

The recorded signals were fed into a 8-bit analog-to-digital converter with a sampling rate of 16 kHz (designed together with V. S. Sopov, Institute of Nuclear Physics, Moscow State University) coupled with a computer via a standard port.

Russian State Medical University, Moscow. ***Address for correspondence:*** al_sokolov@memo.ru. Sokolov A. V.

0007-4888/01/1321-0650$25.00

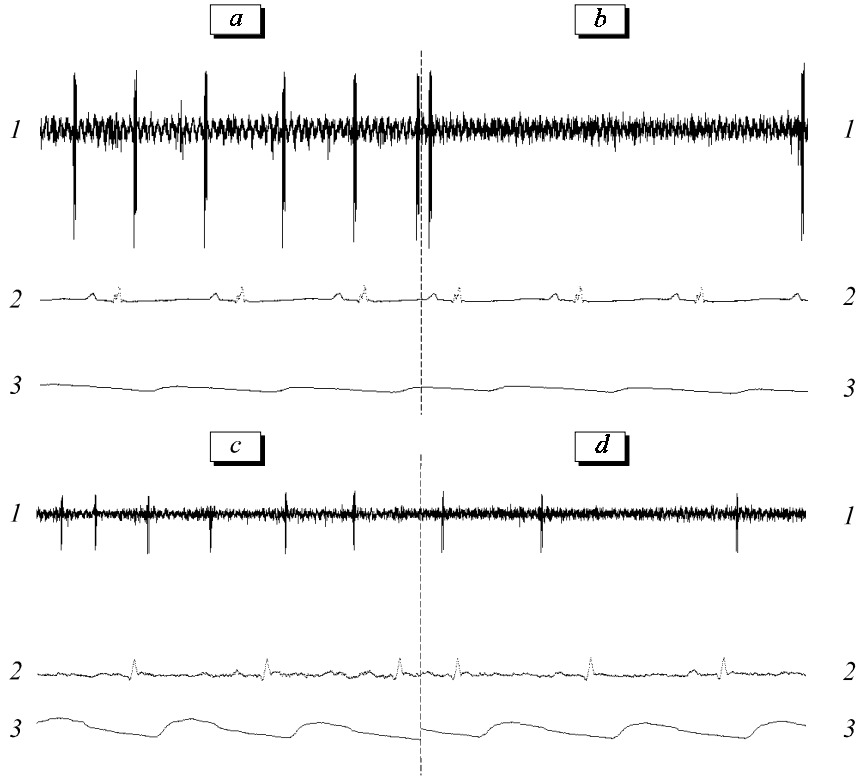

Fig. 1. Impulse activity of sensory neuron (*a*, *b*) and interneuron (*c*, *d*). Baseline activity (*a*, *c*) and 30 sec after the start of DAGO infusion (*b*, *d*). *1*) neurogram; *2*) ECG; *3*) blood pressure; time scale: 2.5 msec per dot.

Functional identification of neurons was performed on the basis of baseline impulse activity [3].

Significant changes ($p<0.05$) in the mean burst firing rate, number of impulses per burst, distribution of bursts throughout the cardiac cycle, and changes in rhythmicity of discharges in respect to the cardiac cycle were evaluated. The test parameters were statistically analyzed using Excel 5.0 by a number of standard tests, including verification of normal distribution, calculation of means, and Student's *t* test for comparing the mean values.

RESULTS

Activity of 34 bulbar cardiovascular neurons (6 afferent neurons and 28 interneurons) was analyzed.

From 3 to 30 sec after the start of DAGO infusion, changes in the discharge pattern were revealed in 83% sensory neurons (Fig. 1, *a*, *b*) and 59% interneurons (Fig. 1, *c*, *d*). By the end of the first minute, responses of all sensory neurons and 75% interneurons were observed: firing rate increased or decreased simultaneously in both types of neurons. Heart rate (HR), systolic and mean dynamic BP did not differ from the baseline values (160.0±4.2 bpm, 152.0±10.1 and 133.0± 5.2 mm Hg, respectively).

Thus, impulse activity of all sensory neurons and the majority of interneurons changed within the first minute of DAGO infusion, *i.e.* under conditions of stable HR and BP.

On the 3rd minute of DAGO infusion, HR decreased by 3.2% of the baseline ($p<0.001$), while BP remained unchanged. Changes in impulse activity were observed in all neurons. At this term impulse activity decreased in most sensory neurons and interneurons. On the 5th minute, a great majority of neurons in both groups increased their firing rates.

On minutes 10-15, systolic BP tended to increase (9.8%, $p=0.12$), although this increase was insignificant. HR (4%, $p<0.001$) decreased by 6.1% on the 15th min ($p<0.01$). The changes in activities of both types of neurons remained co-directed throughout this interval (Fig. 2).

Changes in neuronal activity observed during the first seconds of DAGO injection, when hemodynamic parameters remained stable, could be related to the effect of DAGO on both peripheral μ-opiate receptors [11] and central μ-opiate receptors in the nucleus tractus solitarius [9,10], due to high permeability of the blood-brain barrier for DAGO. Direct effects of DAGO on central receptors were recently demonstrated: the antiarrhythmic effect of DAGO persisted during the blockade of vagal afferent fibers and disappeared after vagotomy (blockade of both afferent and efferent fibers) [4].

Thus, impulse activity of sensory neurons and interneurons during DAGO infusion underwent parallel

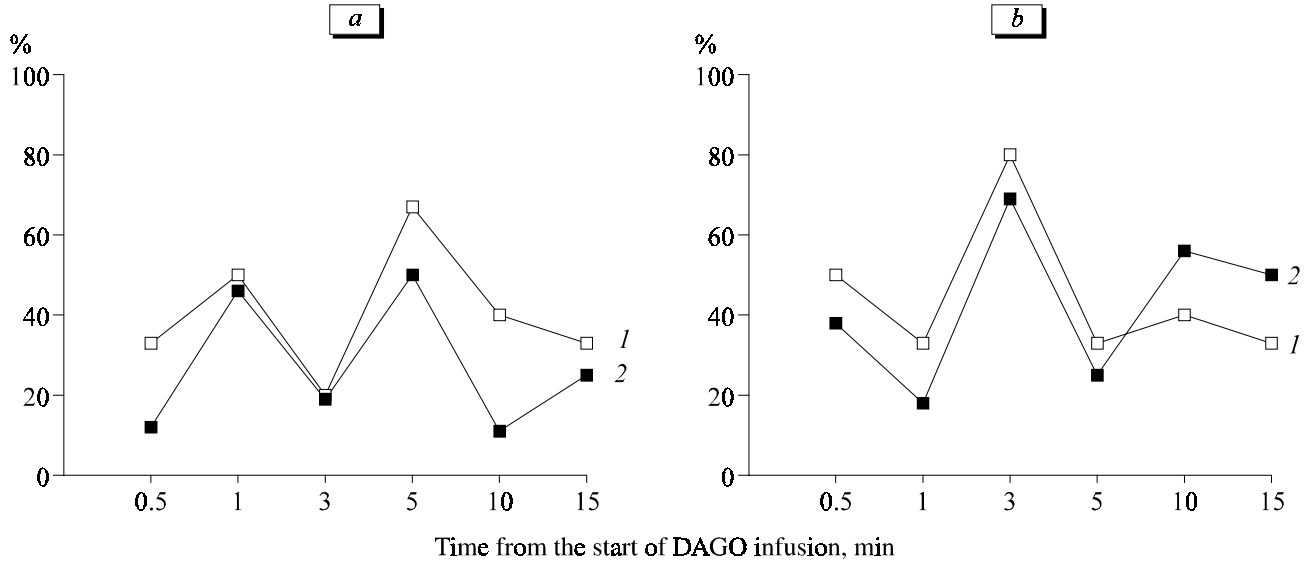

Fig. 2. The number of sensory neurons (*1*) and interneurons responded (*2*) to intravenous DAGO infusion by increased (*a*) or decreased (*b*) firing rate.

changes (Fig. 2). Previous experiments showed that parallel changes in impulse activity of bulbar cardiovascular sensory neurons and interneurons are typical of myocardial ischemia not associated with irreversible ventricular fibrillation. And, on the contrary, opposite changes in impulse activity of these neurons during coronary occlusion culminated in animal death [2].

These findings confirm the hypothesis that the antiarrhythmic effect of μ-opiate receptor agonist DAGO during myocardial ischemia is mediated by its direct influence on neurons of the bulbar cardiovascular center.

REFERENCES

1. A. A. Gratyn', *Modern Problems of Pharmacology of Reticular Formation and Synaptic Transmission* [in Russian], Leningrad (1963) pp. 165-186.
2. G. I. Kositskii, S. D. Mikhailova, S. L. Gorozhanin, and T. M. Semushkina, *Byull. Eksp. Biol. Med.*, **104**, No. 9, 263-265 (1987).
3. G. I. Kositskii, S. D. Mikhailova, S. V. Putygin, *et. al.*, *Current Problems in Experimental Cardiology*, Ed. G. I. Kositskii [in Russian], Moscow (1987) pp. 12-20.
4. S. D. Mikhailova, N. A. Bebyakova, and T. M. Semushkina, *Byull. Eksp. Biol. Med.*, **126**, No. 10, 377-379 (1997).
5. S. D. Mikhailova, A. V. Sokolov, and T. M. Semushkina, *Ibid*, **127**, No. 4, 374-377 (1997).
6. H. Bachelard and M. Pitre, *Br. J. Pharmacol.*, **115**, No. 4, 613-621 (1995).
7. A. Y. Lee and T. M. Wong, *Neurosci. Lett.*, **80**, No. 3, 289-292 (1987).
8. S. W. Rabkin, *Life Sci.*, **45**, No. 12, 1039-1047 (1989).
9. M. R. Dashwood, J. R. Muddle, K. M. Spyer, *Eur. J. Pharmacol.*, **155**, Nos. 1-2, 85-92 (1988).
10. N. Sales, D. Riche, B. P. Roques, and M. Denavit-Saubie, *Brain Res.*, **344**, No. 2, 382-386 (1985).
11. Y. W. Scott, J. K. Wamsley, and V. A. Zarbin, *Science*, **210**, No. 3, 76-78 (1980).