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INCREASE OF CARDIODEPRESSANT ACTIVITY IN MEDIUM INCUBATING THE POSTERIOR PITUITARY LOBE IN SITU DURING VAGAL NERVE STIMULATION IN RAT.

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Previous studies have indicated that there is a cardiodepressant factor in the medium incubating the posterior pituitary lobe *in situ*.

The cardiodepressant activity of the medium incubating the posterior pituitary lobe before and during stimulation of the vagus nerves was tested on isolated auricles of the right heart atrium of a two-day-old rat. It was found that the medium incubating the posterior pituitary lobe collected before stimulation decreased the contraction rate of the auricle by 34%, while that collected during the intermittent stimulation of the central ends of the cut vagus nerves caused a decrease of the auricle contractions frequency by 52%. The addition of cholinergic, serotoninergic, histaminergic receptor blockers or prostaglandin synthetase into Ringer-Lock's solution bathing the auricle has no effect on the changes of the contraction rate caused by the incubation medium.

Key words: *cardiodepressant factor, posterior pituitary lobe, afferent fibres of vagus nerve, auricle of the heart atrium.*

INTRODUCTION

Chang et al. (1) demonstrated that stimulation of the central end of the cut vagus nerve caused the release of pressor substance (pituitrin) from the posterior pituitary lobe. Mills and Wang (2, 3) reported that such stimulation induced antidiuretic response, which was associated with the enhancement of vasopressin release from the posterior pituitary lobe. Further studies showed that the release of vasopressin and oxytocin from the posterior pituitary lobe during vagus nerves stimulation was associated with the increase of bioelectric activity of the neurons of the hypothalamus supraoptic and paraventricular nuclei (4, 5).

The vasopressin release from the posterior pituitary lobe during stimulation of the vagus nerves takes place through a cholinergic transmitter (1, 6, 7, 8, 9). It has also been demonstrated that acetylcholine stimulates the supraoptic nucleus neurons and increases vasopressin release through cholinergic nicotine receptors in the hypothalamus (10), and muscarine receptors in the posterior pituitary lobe (9).

The aim of the present studies was to elucidate if stimulation of vagal afferent fibres, which release vasopressin and oxytocin from the posterior pituitary lobe would also increase the release of a cardiodepressant factor.

MATERIAL AND METHODS

Experimental animals and collection of the medium incubating the posterior pituitary lobe in situ.

The experiments were performed on 10 male rats 300—320 g body weight, being the F_1 generation of cross-breeding of August strain males and Wistar strain females, from the stock of the Institute of Oncology in Warsaw. The animals were anaesthetized by the intraperitoneal injection of a solution containing 6 mg of chloralose and 60 mg of urethane per 100 g of body weight. The right and left vagus nerves were exposed on the neck and a polyethylene tube was introduced into the femoral artery and connected with a mercury manometer (11) in order to measure mean arterial blood pressure. The slightly modified technique of Worthington (12) of transpharyngeal approach to expose the pituitary was used.

The removal of the anterior lobe and the incubation of the posterior lobe *in situ* were performed according to the method of Traczyk (13) with some later changes (14). The posterior pituitary lobe was incubated in McIlwain and Rodnight's solution containing in mmol/l H_2O: NaCl-120, KCl-4.8, KH_2PO_4-1.2, $MgSO_4$-1.3, $CaCl_2$-2.8, $NaHCO_3$-26.0, glucose-10.0. The incubation fluid in which the posterior pituitary lobe was immersed was sucked with the use of a water vaccum pump into a tube containing methanol (2.5 ml methanol to 1 ml of the incubation medium). The first sample of the incubation medium was colleceted during 31—60 min. and the second sample during 61—90 min from onset of incubation in situ. During second sample collection the intermittent stimulation of the central ends of both vagus nerves was performed. After the precipitation of proteins each fluid sample was centrifuged at 10 000 g and 0.1 ml was taken to determine the peptide concentration. Direct peptide determination was performed by measurement of absorption at 230 nm (for peptide bond absorption) using a VSU 2-P Spectrophotometer Carl Zeiss, Jena). The supernatant was lyophilized and stored until bioassay.

During stimulation vagus nerves the mean arterial blood pressure was recorded by a mercury manometer (11). The respiratory rate was counted and expressed as breath per min. before and after the vagus nerve stimulation.

Stimulation of vagus nerves

Vagus nerves were separated from the surrounding tissues in the neck just before the stimulation, cut and laid on bipolar silver electrodes. The electrodes were connected to a Grass stimulator model S4K. The nerves were moistened with 0.9% NaCl solution.

They were stimulated by monophasic electric impulses of 60 Hz, 2 msec and 10V. The cut vagus nerves were stimulated alternatively through a commutator with 1.5 sec on and 1.5 sec off breaks for 30 minutes during the incubation of the posterior pituitary lobe in situ. The electric pulses were monitored on a ST-509A oscilloscope (Radiotechnika, Wrocław).

Determination of cardiodepressant activity

The cardiodepressant activity was determined on the isolated auricle of the right heart atrium of a two-day-old rat according to the previously described method (15). Briefly, two-day-old rats were decapitated, the heart was isolated, the right auricle of the right heart atrium was prepared and put into a chamber of 100 μl volume of Ringer-Lock solution containing in mmol/l H_2O: NaCl — 153.0, KCl — 5.6, $CaCl_2$ — 3.3, $NaHCO_2$ — 1.7, glucose — 5.5. The solution was oxygen-saturated and flowed at a rate of 50 μl/20 sec. The auricle started to contract spontaneously at a constant rhythm after 15—30 min.

Lyophilized 30 min. samples of the fluid incubating the posterior pituitary lobe were dissolved in 0.5 ml 0.9% NaCl and injected into the chamber in the volume of 20 μl. The contraction of the auricle before and after the introduction of the tested sample was counted with the use a stereomicroscope. Thirty successive contractions of the auricle was counted 4 times before and 8 times after the introduction of every sample and averaged per sec. Each sample was tested on 6 auricle. The determination of cardiodepressant activity was repeated with atropine sulphate 5×10^{-6} mol/l (Boehringer Ingelheim), methysergide maleate 5×10^{-7} mol/l (Sandoz Pharmaceuticals bath No. 98603), pyrilamine maleate 10^{-5} mol/l (Ruger bath No. 7125), indomethacin 3×10^{-6} mol/l (Merck Sharp and Dohme Research Lab., bath No. L-590) added to the Ringer-Lock solution.

The results are expressed as a percentage of the control frequency of auricle contractions. Means$\pm$SE are reported. Student's unpaired t — test was used for statistical analysis. $P < 0.001$ is considered significant.

RESULTS

The effectiveness of stimulation of the centropetal ends of vagus nerves was estimated on the basis of the arterial blood pressure and respiratory rate. The mean arterial blood pressure before stimulation was 22.7 ± 1.3 kPa (170 ± 10 mm Hg) ($n = 10$), while after the start of stimulation it decreased to 14.7 ± 1.6 kPa (110 ± 12 mm Hg) ($n = 10$) and returned during 15—20 min to the initial value.

Before stimulation the respiratory rate was 34 ± 3 per min. ($n = 10$) at the beginning of stimulation it decreased and was 20 ± 2 inspirations per min. ($n = 10$).

The peptide concentration in the fluid samples collected before the vagus nerves stimulation corresponded to 1.93 ± 0.09 mg of peptides per ml ($n = 10$), while in the samples collected during stimulation the concentration was significantly higher 3.98 ± 1 mg of peptides per ml ($n = 10$).

The fluid incubating the posterior pituitary lobe in situ collected before

the vagus nerves stimulation caused a decrease of frequency of the auricle contraction rate of about 34%±5% ($P < 0.001$, $n = 10$), while the fluid collected during the vagus nerves stimulation decreased the contraction frequency by about 52%±4% ($P < 0.001$, $n = 10$). Mean difference between cardiodepressant activity of the fluid incubating the posterior pituitary lobe before stimulation vagus nerves and during stimulation is statistically significant ($P < 0.01$).

Atropine blockade of muscarine receptors in the auricle did not abolish the cardiodepressant activity of the fluid incubating the posterior pituitary lobe. The fluid caused a decrease of contraction rate by 38%±4% ($P < 0.001$, $n = 6$) collected before stimulation and 51%±3% ($P < 0.001$, $n = 6$) during stimulation (Fig. 1).

It was observed that the fluid collected before the vagus nerves stimulation and after pyrilamine maleate, methysergide maleate blocking of histaminergic, serotoninergic receptors caused a decrease of contraction rate by

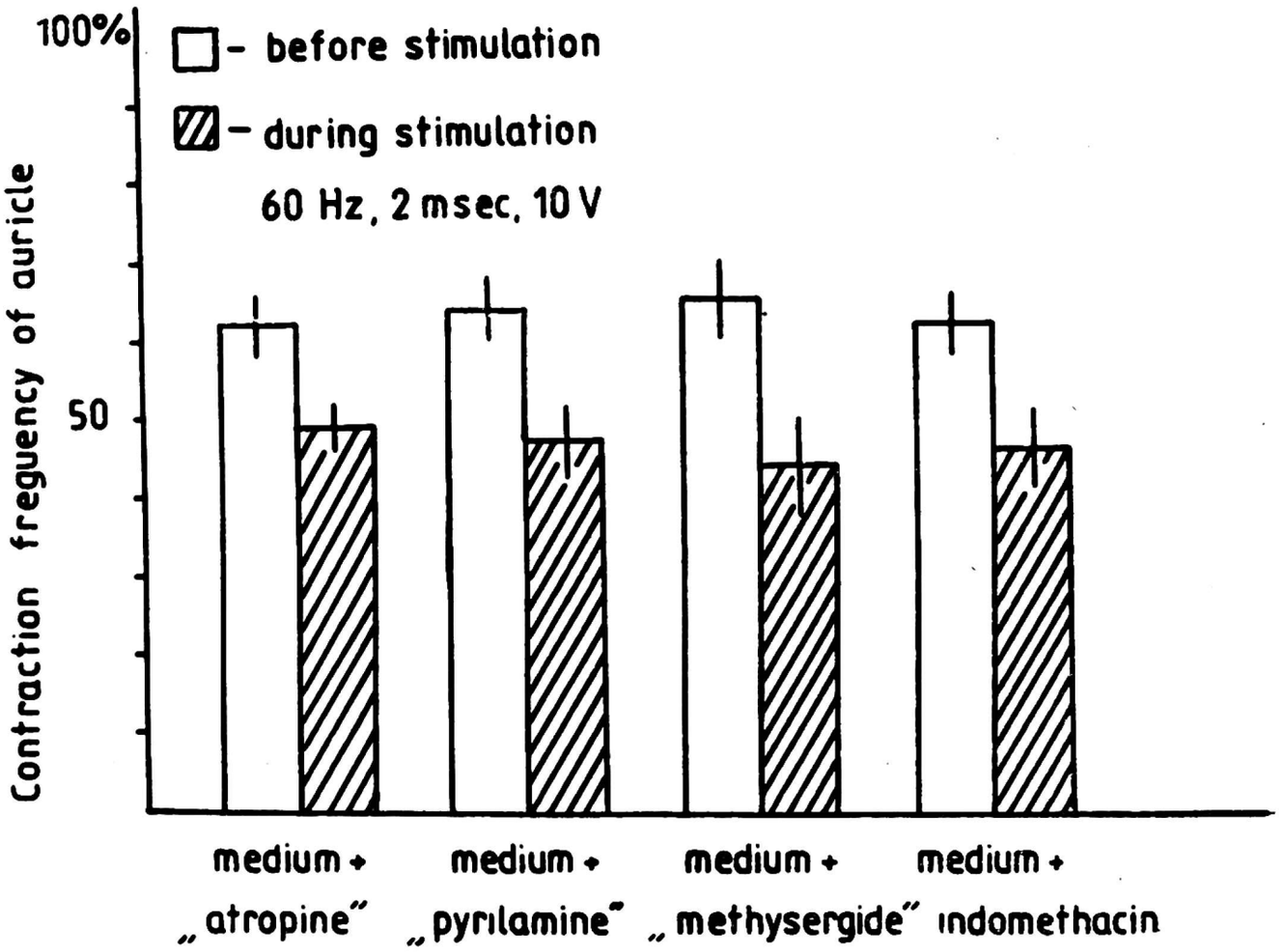

Fig. 1. Contraction rate of an isolated auricle under the influence of the medium incubating the posterior pituitary lobe before and during vagal nerve stimulation, and after addition of pyrilamine, methysergide and indomethacin to the medium perfusing the auricle. 100% — contraction frequency of isolated auricle (4×30 successive contraction) counted before the introduction of the medium incubating the posterior pituitary lobe.

36%±4% ($P < 0.001$, $n = 6$) and 35%±5% ($P < 0.001$, $n = 6$), respectively. The fluid collected during the stimulation and after blocking histaminergic serotoninergic receptors decreased contraction rate by 53% ±5% ($P < 0.001$, $n = 6$) and 56%±6% ($P < 0.001$, $n = 6$), respectively.

The fluid collected before the vagus nerves stimulation and after the blocade of prostaglandin synthetase induced a decrease of contraction rate by 38%±4% ($P < 0.001$, $n = 6$) and during the stimulation and after the prostaglandin synthetase blocade caused a decrease by 54%±5% ($P < 0.001$, $n = 6$) (*Fig. 1*).

DISCUSSION

The previous reports presented results indicating that a cardiodepressant factor is released into the medium incubating *in situ* the posterior pituitary lobe (16). It was also determined that it is probably a low-molecule compound (m. w. ca 1000) with peptide structure, that causes the negative chronotropic effect on an isolated auricle of the right heart atrium of two-day-old rats (17).

The results of the present experiments indicate that the medium incubating *in situ* the posterior pituitary lobe of the rat, collected during intermittent stimulation of the vagus nerves caused more intensive release of peptides into the incubating medium and a more pronounced reduction of the contraction rate of the auricle than the medium collected prior to vagal nerves stimulation.

The increased release of the cardiodepressant factor from the posterior pituitary lobe into the incubating medium during vagal nerve stimulation may be associated with a similar mechanisms as vasopressin release (18, 2, 3).

It was demonstrated using the method of posterior pituitary lobe incubation, that vasopressin is present in the samples of the incubating medium (13). Mills and Wang (2, 3) observed that electrical stimulation of the vagal nerve caused an increase of antidiuretic hormone release from the posterior pituitary lobe. Numerous authors demonstrated that i. v. vasopressin infusion may reduce the heart rate (19, 20). Osborn et al. (20) observed also that vasopressin-induced bradycardia in rats is associated with the reduction of tonic activity of sympathetic fibres or with the increase of tonic activity of vagal nerve fibres running to the heart.

Other investigators demonstrated in experiments on isolated rat heart that vasopressin induces contraction of the coronary blood vessels, which leads to myocardial hypoxia and reduction of the systolic rate (21). However, the experiments performed by Lefer and Inge (22) on an isolated

papillary muscle demonstrated that vasopressin in concentrations ranging from 10^{-8} mol/l to 10^{-5} mol/l has no cardiodepressant effect. Similarly, in our study vasopressin had no effect on the contraction rate of an isolated auricle (15).

It follows from our studies and those reported by others that cardiodepressant activity of the medium incubating the posterior pituitary lobe is not associated with vasopressin.

The nerve endings in the posterior pituitary lobe are known to store and release into the blood stream not only vasopressin and oxytocin, but also acetylcholine (23). In the present study the increase of cardiodepressant activity was not caused by acetylcholine release, because addition of atropine to the Ringer-Lock solution perfusing the auricle did not abolish the cardiodepressant activity of the medium incubating the posterior pituitary lobe.

It was observed that reduction of the contraction rate of the isolated auricle was not due to histamine, serotonin or prostaglandins, because the addition of pyrilamine methysergide and indomethacin to the fluid perfusing the isolated auricle did not change the cardiodepressant activity of the tested medium incubating the posterior pituitary lobe. Moreover, histamine, serotonin and prostaglandins are known increase the contraction rate of an isolated atrium and isolated rat heart (24, 25, 26).

It should also be taken into account that nucleotides may be released into the medium incubating the posterior pituitary lobe. Gordon (27), and Gordon and Hesse (28) demonstrated that i. v. injection of ADP (adenosinodiphosphate), adenosine, AMP (adenosinomonophosphate), ATP (adenosinotriphosphate) causes a decrease of arterial blood pressure as well as a reduction of heart rate. Schally et al., (29) fractionating extracts from bovine, ovine and pig hypothalami isolated also a fraction reducing arterial blood pressure. The authors suggest that the vasodepressant activity of the extracts from the hypothalami was caused by adenosinomonophosphate present in the extracts. Other authors in their experiments on heart cell cultures observed that cAMP (cyclic adenosinomonophosphate), cGMP (cyclic guanosinomonophosphate) and GTP (guanosinotriphosphate) have no effect on the contraction rate of myoblasts. However, dibutyrate of cGMP (dbcGMP) decreased the contraction rate of myoblasts only by 15%, whereas dibutyrate cAMP (dbcAMP) increased the contraction rate by 25% (30).

Experiments performed on an isolated auricle of the right atrium of a two-day-old rat demonstrated that ADP, ATP caused only in concentrations of 1 mol/l and 10 mol/l, a reduction of auricle contraction frequency by 28% and 35%, respectively, whereas cAMP and dbcGMP

had no influence on the contraction rate (17). Such high concentrations of nucleotides do not occur in the organism.

During circulatory shock various bioactive substances are also released into the blood stream. Among them MDF (Myocardial Depressant Factor), discovered in the blood of cats in post-haemorrhagic shock by Brand and Lefer (31), is the best known cardioactive factor. MDF is a peptide or glucopeptide with m. w. 500—1000 (32), which has a negative inotropic effect on isolated papillary muscle (33, 34).

Numerous experiments indicated the presence of an atrial natriuretic factor (ANF) in the posterior pituitary lobe of animals. This factor has natriuretic, diuretic and hypotensive effects (35). Experiments on an isolated right atrium proved that ANF has no inotropic and chronotropic effect in the used concentration (36).

The present experiments demonstrate that the medium incubating the posterior pituitary lobe „in situ", collected during vagal nerve stimulation has an increased cardiodepressant activity in comparison with the samples collected before vagal nerve stimulation. The cardiodepressant activity was not due to acetylcholine, histamine, serotonin and prostaglandins.

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REFERENCES

1. Chang HC, Chia KF, Hsü CH, Lim RKS. Reflex secretion of the posterior pituitary elicited through the vagus. *J Physiol* (London) 1937; 90: 87P—89P.
2. Mills E, Wang SC. Liberation of antidiuretic hormone: location of ascending pathways. *Am J Physiol* 1964; 207: 1399—1404.
3. Mills E, Wang SC. Liberation of antidiuretic hormone: pharmacologic blocade of ascending pathways. Am *J Physiol* 1964; 207: 1405—1410.
4. Dyball REJ, Koizumi K. Electrical activity in the supraoptic and paraventricular nuclei associated with neurohypophysial hormone release. *J Physiol* (London) 1969; 201: 711—722.
5. Barker JL, Crayton JW, Nicoll RA. Antidromic and orthodromic responses of paraventricular and supraoptic neurosecretory cells. *Brain Res* 1971; 33: 353—366.
6. Pickford M. The inhibitory effect of acetylcholine on water diuresis in the dog and its pituitary transmission. *J Physiol* (London) 1939; 95: 226—238.
7. Pickford M. The action of acetylcholine in the supraoptic nucleus of the chloralosed dog. *J Physiol* (London) 1947; 106: 264—270.

8. Gosbee JL, Lederis K. *In vivo* release of antidiuretic hormone by direct application of acetylcholine or carbachol to the rat neurohypophysis. *Can J Physiol Pharmacol* 1972; 50: 618—620.
9. Gregg CM, Sladek CD. A compartmentalized organ-cultured hypothalamo-neurohypophyseal system for the study of vasopressin release. *Neuroendocrinology* 1984; 38: 397—402.
10. Sladek CD, Joynt RJ. Characterization of cholinergic control of vasopressin release by the organ cultured rat hypothalamo-neurohypophyseal system. *Endocrinology* 1979; 104: 659—663.
11. The Pharmacopeia of the United States of America, *Sixteenth Revision*, October 1, 1960; 851.
12. Worthington WC, Jr. Blood samples from the pituitary stalk of the rat: method of collection and factors determining volume. *Nature* 1966; 210; 710—712.
13. Traczyk WZ. Vasopressin release from incubated in situ posterior pituitary lobe *in rats*. *Bull Acad Pol Sci Ser Sci Biol* 1972; 20: 351—356.
14. Cyrkowicz A, Traczyk WZ. Effect of intravenous and intracarotid injections of hypertonic fluids on the release of melanocyte-stimulating hormone from the rat posterior pituitary lobe incubated *in situ*. *J Endocr* 1975; 66: 85—91.
15. Gorąca A, Traczyk WZ, Konarzewska A. The influence of amino acids, vasopressin and oxytocin on spontaneous contraction of the right auricle of the right atrium of two-day-old rats *in vitro*. *Acta Physiol Pol* 1984; 35: 454—459.
16. Gorąca A, Tranda T, Traczyk WZ. Appearance of a cardiodepressant factor in the medium incubating *in situ* the posterior pituitary lobe of the rat. I. Isolation of active compound. *Acta Physiol Pol* 1984; 35: 460—468.
17. Gorąca A, Traczyk WZ. Appearance of a cardiodepressant factor in the medium incubating in situ the posterior pituitary lobe of the rat. II. Differentation from other active compounds. *Acta Physiol Pol* 1988; 39: 98—111.
18. Gregg CM. The compartmentalized hypothalamo-neurohypophyseal system. Evidence for a neurohypophyseal action of acetylcholine on vasopressin release. *Neuroendocrinology* 1985; 40: 423—429.
19. Emerson TE, Jr. Effects of angiotensin, epinephrine, norepinephrine and vasopressin on venous return. Am *J Physiol* 1966; 210: 933—942.
20. Osborn JW, Jr, Skelton MM, Cowley AW, Jr. Hemodynamic effects of vasopressin compared with angiotensin II in conscious rats. Am *J Physiol* 1987; 252: H628—H637.
21. Boyle WA, Segel LD. Direct cardiac effects of vasopressin and their reversal by a vascular antagonist. Am *J Physiol* 1986; 251: H734—H741.
22. Lefer AM, Inge TF, Jr. Differentation of a myocardial depressant factor present in shock plasma from known plasma peptides and salts. *Proc Soc Exp Biol Med* 1973; 142: 429—433.
23. Lederis K, Livingston A. Acetylcholine and related enzymes in the neural lobe and anterior hypothalamus of the rabbit. *J Physiol* (London) 1969; 201: 695—709.
24. Trendelenburg U. The action of histamine and 5-hydroxytryptamine on isolated mammalian atria. *J Pharmacol Exp Ther* 1960; 130: 450—460.
25. Laher I, McNeill JH. Effects of histamine on rat isolated atria. Can *J Physiol Pharmacol* 1980; 58: 1114—1116.
26. Shaffer JE, Malik KU. Mechanism of action of arachidonic acid in the isolated perfused rat heart. Can *J Physiol Pharmacol* 1984; 62: 551—558.
27. Gordon DB. Basis of the depressor action of adenosine phosphate. Am *J Physiol* 1961; 201: 1126—1130.

28. Gordon DB, Hesse DH. Blood pressure lowering action of adenosine diphosphate and related compounds. Am *J Physiol* 1961; 201: 1123—1125.
29. Schally AV, Kastin AJ, Barrett JF, Carter WH, Bowers CY, White WF. On vasodepressor activity in the hypothalamus. *Biochem Pharmacol* 1966; 15: 1805—1811.
30. Krause EG, Halle W, Wollenberger A. Effect of dibutyryl cyclic GMP on culture beating rat heart cells. In: Advances in Cyclic Nucleotide Research, Vol. 1. Physiology and Pharmacology of cyclic AMP, edited by P. Greengard R. Paoletti and GA Robison, *Raven Press*, New York, 1972; 301—305.
31. Brand ED, Lefer AM. Myocardial depressant factor in plasma from cats in irreversible post-oligemic shock. *Proc Soc Exp Biol Med* 1966; 122: 200—203.
32. Lefer AM, Martin J. Origin of myocardial depressant factor in shock. Am J Physiol 1970; 218: 1423—1427.
33. Lefer AM. Role of a myocardial depressant factor in the pathogenesis of circulatory shock. *Fed Proc* 1970; 29: 1836—1847.
34. Lefer, AM. Interaction between myocardial depressant factor and vasoactive mediators with ischemia and shock. Am *J Physiol* 1987; 252: R193—R205.
35. de Bold AJ, Borenstein HB, Veress AT, Sonnenberg H. A rapid and potent natriuretic response to intravenous injections of atrial myocardial extract in rats. *Life Sci* 1981; 28: 89—94.
36. Hiwatari M, Satoh K, Angus JA, Johnston CI. No effect of atrial natriuretic factor on cardiac rate, force and transmitter release. *Clin Exp Pharmacol Physiol* 1986; 13: 163—168.

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