

# Effect of Endotoxin on the S-A Node *in Situ* in the Dog Heart<sup>1</sup> (35803)

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(Introduced by E. G. Erdös)

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Although injection of endotoxin has a potent effect on the peripheral circulation, there is no evidence available to show that it would influence heart function significantly. In 1960, Gilbert (1) reviewed the hemodynamic effects of endotoxin and indicated that the heart is not entirely immune from the effects of endotoxin. Several reports have been published on myocardial function and endotoxin (2-5) but the effect on pacemaker activity of the S-A node has not been studied selectively. The present studies were undertaken to evaluate the effect of endotoxin on the S-A node of dogs *in situ*, using the direct perfusion method of the sinus node artery (6, 7).

**Methods.** Six mongrel dogs of either sex, weighing 11 to 16 kg, were anesthetized with sodium pentobarbital, 30 mg/kg, intravenously. Artificial respiration was maintained with a Harvard respirator. The direct perfusion technique of the sinus node artery in these experiments was originally devised by James and Nadeau (6) and modified by Hashimoto *et al.* (7, 8). Systemic blood pressure in the femoral artery was measured continuously with a Grass polygraph recorder. The heart rate was calculated from P-P intervals in the EKG recordings (Lead II). Sodium heparin, 500 U/kg, was given at the beginning of the perfusion and 200 U/kg were added at 1-hr intervals. Both vagi were cut at the middle of the neck. A bipolar platinum electrode was applied at the distal

end of the right vagus nerve for electric stimulation. Supramaximal stimuli (5 to 10 V, 1 msec, 30 cps) were applied for 5 sec by means of a square wave stimulator. Endotoxin (Difco, *Escherichia coli*) in a 5% glucose solution was injected intra-arterially into the sinus node artery with a micro-injector at a rate of 0.01 to 0.05 ml/4 sec or with an infusion pump (Harvard infusion apparatus) at a rate of 0.1 to 0.5 ml/min. Drugs used were acetylcholine chloride, DL-norepinephrine hydrochloride, atropine sulfate and DL-propranolol hydrochloride.

**Results. A. Influence of endotoxin on sinus rhythm.** Endotoxin at a dose level of 1 to 30  $\mu$ g/4 sec did not have any chronotropic effect. Larger doses of endotoxin, 100  $\mu$ g to 1 mg, induced a slightly negative chronotropic response as shown in Table I. This negative effect was not blocked by 10  $\mu$ g of atropine. An intra-arterial (ia) injection of endotoxin at doses of 10  $\mu$ g to 1 mg did not cause hypotension. When more than 3 mg of endotoxin was given in continuous ia infusion, it lowered the mean systemic arterial blood pressure by about 10 to 20 mm Hg that lasted for 5 to 10 min.

**B. Endotoxin and the effect of acetylcholine.** Electric vagal stimulation induced a negative chronotropic response. The selective injection of acetylcholine in doses of 1 to 3  $\mu$ g into the sinus node artery also induced a negative chronotropic response (Table II). These effects were not blocked by ia pretreatment with 100  $\mu$ g to 1 mg of endotoxin or by ia continuous infusion of endotoxin.

**C. Endotoxin and the effect of norepinephrine.** Injection of norepinephrine, 0.1 to 1  $\mu$ g, into the sinus node artery produced a prominent positive chronotropic response. This response was not significantly blocked by large amounts of endotoxin, ranging from

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TABLE I. Chronotropic Response to Endotoxin Injected into the Sinus Node Artery in the Dog *in Situ*.<sup>a</sup>

Dose of endotoxin	No. of dogs	Initial HR (beats/min)	Negative chronotropic effect (% decrease)
10 $\mu$ g	6	166 $\pm$ 3.2	0
30 $\mu$ g	6	166 $\pm$ 3.5	2.0 $\pm$ 1.2
100 $\mu$ g	6	171 $\pm$ 2.0	3.2 $\pm$ 1.4
300 $\mu$ g	5	173 $\pm$ 4.7	10.0 $\pm$ 2.5
1 mg	2	166	13.5

<sup>a</sup> Results are given as mean  $\pm$  SE; HR = heart rate.

100  $\mu$ g to 1 mg, as shown in Table III. The chronotropic effect induced by norepinephrine, however, was inhibited by 1 to 10  $\mu$ g of propranolol.

**Discussion.** There have been several reports on the effects of endotoxin on the heart (2-5, 9, 10, 12). Siegel and Fabian (3) reported that endotoxin produced a primary myocardial depression which was independent of the fall of aortic blood pressure in the dog. Vargas and Beck (4) observed that endotoxin did not depress the isolated perfused rabbit heart. Weil *et al.* (9) showed that the canine heart tested with endotoxin is capable of normal function with maintenance of cardiac output as long as the blood supply to the right atrium is maintained constant. On the other hand, Kutner and Cohen (2) demonstrated that endotoxin did not affect the contractility of the isolated papillary muscle in the cat. More recently, Hinshaw *et al.* (5) perfused an isolated heart and lungs removed from a donor dog with venous blood from an intact animal to see the effects of endotoxin on the heart. They found no evidence of a direct toxic action of endotoxin on myocardial tissue at dose levels that induced shock.

We have observed an effect of endotoxin

on S-A pacemaker activity using the direct *in situ* perfusion method of the canine sinus node artery. The threshold dose for a negative chronotropic response to endotoxin was 30  $\mu$ g. The higher dose of 100  $\mu$ g to 1 mg induced a more pronounced negative chronotropic response. Hashimoto *et al.* (8) have shown the flow rate of the sinus node perfusion to be about 2 ml/min, so 10 to 30  $\mu$ g of endotoxin injected into the sinus node artery is comparable to a plasma concentration that can produce shock. This level did not affect the S-A node. In addition to studying the effects of endotoxin on the S-A node, we investigated the actions of endotoxin on the nervous elements and on acetylcholine and norepinephrine. In 1969, Hashimoto and Chiba (11) showed that tetrodotoxin, 1 to 3  $\mu$ g, injected into the sinus node artery blocked the effect of electric stimulation of the vagus nerve. In our experiments, however, application of large amounts of endotoxin, 100  $\mu$ g to 1 mg, failed to block a negative chronotropic response to electric stimulation of the vagus nerve.

In 1970, Bhagat *et al.* (12) reported that the isolated atrial tissue from endotoxin-treated guinea pigs had a reduced sensitivity to norepinephrine. We did not observe such

TABLE II. Absence of Action of Endotoxin on the Effect of Vagal Stimulation and of Acetylcholine (ACh) Injected into the Sinus Node Artery.

	No. of dogs	Initial HR (beats/min)	Negative chronotropic effect (%)	
			Control	After 100 $\mu$ g to 1 mg of endotoxin
Effect of vagal stimulation	5	164 $\pm$ 3.8	37 $\pm$ 8.8	34 $\pm$ 9.1
ACh, 1 $\mu$ g	5	167 $\pm$ 2.9	25 $\pm$ 11.5	21 $\pm$ 14.7
3 $\mu$ g	3	166 $\pm$ 10.3	31 $\pm$ 2.1	37 $\pm$ 10.2

TABLE III. Lack of Effect of Endotoxin on the Positive Chronotropic Response to Norepinephrine.

Dose of norepinephrine ( $\mu\text{g}$ )	No. of dogs	Initial HR (beats/min)	Positive chronotropic effect (%)	
			Control	After 100 $\mu\text{g}$ to 1 mg of endotoxin
0.3	5	152 $\pm$ 9.2	29 $\pm$ 7.4	28 $\pm$ 5.6
1.0	3	163 $\pm$ 7.1	47 $\pm$ 3.2	39 $\pm$ 12.1

effect of endotoxin on the S-A node of the dog. Furthermore, we confirmed that endotoxin did not modify the effect of acetylcholine injected into the sinus node artery as shown in Table II.

Thus we conclude that endotoxin does not affect the pacemaker activity and does not modify the autonomic nervous mechanism at a dose level sufficient to induce shock.

*Summary.* Concentrations of endotoxin equivalent to those producing shock when given intravenously did not affect the S-A pacemaker activity of the dog *in situ*. Larger amounts of endotoxin, 100  $\mu\text{g}$  to 1 mg, directly induced a negative chronotropic response, which was not blocked by atropine treatment. Endotoxin did not influence the effect of vagal stimulation and responses to acetylcholine and norepinephrine injected into the sinus node artery.

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1. Gilbert, R. P., *Physiol. Rev.* **40**, 245 (1960).
2. Kutner, F. R., and Cohen, J., *J. Surg. Res.* **6**, 83 (1966).
3. Siegel, J. H., and Fabian, M., *J. Amer. Med. Ass.* **200**, 696 (1967).
4. Vargas, R., and Beck, L., *Fed. Proc., Fed. Amer. Soc. Exp. Biol.* **16**, 342 (1957).
5. Hinshaw, L. B., Archer, L. T., Miller, J. A., Greenfield, L. J., and Guenter, C. A., *Physiologist* **13**, 222 (1970).
6. James, T. N., and Nadeau, R. A., *Henry Ford Hosp. Med. Bull.* **10**, 21 (1962).
7. Hashimoto, K., Tanaka, S., Hirata, M., and Chiba, S., *Circ. Res.* **21**, 297 (1967).
8. Hashimoto, K., Chiba, S., Tanaka, S., Hirata, M., and Suzuki, H., *Amer. J. Physiol.* **215**, 1183 (1968).
9. Weil, M. H., Machean, L. D., Visscher, M. B., and Spink, W. W., *J. Clin. Res.* **35**, 1191 (1956).
10. Leese, C. E., Poel, W. E., and Berman, H., *Fed. Proc., Fed. Amer. Soc. Exp. Biol.* **9**, 76 (1950).
11. Hashimoto, K., and Chiba, S., *J. Pharmacol. Exp. Ther.* **170**, 91 (1969).
12. Bhagat, B., Cavanagh, D., Merrild, B. N., Rana, M. W., and Rao, P. S., *Brit. J. Pharmacol.* **39**, 688 (1970).

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