

UNIVERSITY OF OKLAHOMA MEDICAL CENTER

AD 746459

11

EFFECT OF ENDOTOXIN ON
AV CONDUCTIVITY IN THE DOG

S. Chiba and T. Nakajima

DDC
REGISTERED
AUG 15 1972
REGISTERED
C

Technical Report No. 53
University of Oklahoma Medical Center THEMIS Contract

This document has been approved for public release
and sale; its distribution is unlimited

Reproduction by
NATIONAL TECHNICAL
INFORMATION SERVICE
U.S. GOVERNMENT PRINTING OFFICE
WASHINGTON, D.C. 20540

Reproduction in whole or in part is permitted for
any purpose of the United States Government

MEDICAL CENTER RESEARCH AND DEVELOPMENT OFFICE
OF THE UNIVERSITY OF OKLAHOMA FOUNDATION, INC.
800 Northeast Thirteenth Street
Oklahoma City, Oklahoma 73104

UNCLASSIFIED

Security Classification

DOCUMENT CONTROL DATA - R & D

(Security classification of title, body of abstract and indexing annotation must be entered when the overall report is classified)

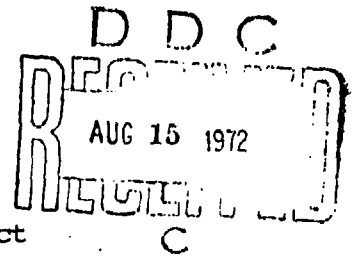
1. ORIGINATING ACTIVITY (Corporate author)		2a. REPORT SECURITY CLASSIFICATION	
MEDICAL CENTER RESEARCH AND DEVELOPMENT OFFICE OF THE UNIVERSITY OF OKLAHOMA FOUNDATION, INC.		UNCLASSIFIED	
2b. GROUP		UNCLASSIFIED	
3. REPORT TITLE			
EFFECT OF ENDOTOXIN ON AV CONDUCTIVITY IN THE DOG			
4. DESCRIPTIVE NOTES (Type of report and, inclusive dates)			
Technical Report			
5. AUTHOR(S) (First name, middle initial, last name)			
S. Chiba, T. Nakajima			
6. REPORT DATE	7a. TOTAL NO. OF PAGES	7b. NO. OF REFS	
June 6, 1972	8	17	
8a. CONTRACT OR GRANT NO.	9a. ORIGINATOR'S REPORT NUMBER(S)		
N00014-68-A-0496	53		
a. PROJECT NO	9b. OTHER REPORT NO(S) (Any other numbers that may be assigned this report)		
NR 105-516			
c.			
d.			
10. DISTRIBUTION STATEMENT			
This document has been approved for publication release and sale; its distribution is unlimited.			
11. SUPPLEMENTARY NOTES		12. SPONSORING MILITARY ACTIVITY	
		Office of Naval Research	
13. ABSTRACT			
<p>Using constant flow perfusion from 4 to 6 ml/min of the AV node artery the effect of endotoxin was investigated in six vagotomized dogs in situ. Preparations readily demonstrated AV block following injection of 1 µg of acetylcholine. AV block was observed in 2 out of 6 dogs at a dose of 1 mg/4 sec, in 1 out of 6 at a plasma concentration of 20 µg/ml and in 2 out of 3 at a plasma concentration of 200 µg/ml. Endotoxin-induced AV block was not influenced by treatment with atropine. Endotoxin pretreatment of 1 mg or 10 or 20 µg/ml did not affect acetylcholine-induced AV block and norepinephrine-induced nodal rhythm.</p>			

EFFECT OF ENDOTOXIN ON
AV CONDUCTIVITY IN THE DOG

S. Chiba and T. Nakajima

Technical Report No. 53
University of Oklahoma Medical Center THEMIS Contract

June 6, 1972



Research sponsored by the Office of Naval Research
Contract N00014-68-A-0496
Project NR 105-516

Reproduction in whole or in part is permitted for
any purpose of the United States Government

This document has been approved for public release
and sale; its distribution is unlimited

MEDICAL CENTER RESEARCH AND DEVELOPMENT OFFICE
OF THE UNIVERSITY OF OKLAHOMA FOUNDATION, INC.

Abstract

Using constant flow perfusion from 4 to 6 ml/min of the AV node artery, the effect of endotoxin was investigated in six vagotomized dogs in situ. All preparations readily demonstrated AV block following injection of 1 μ g of acetylcholine. AV block was observed in 2 out of 6 dogs at a dose of 1 mg/4 sec, in 1 out of 6 at a plasma concentration of 20 μ g/ml and in 2 out of 3 at a plasma concentration of 200 μ g/ml. Endotoxin-induced AV block was not influenced by treatment with atropine. Endotoxin pretreatment of 1 mg or 10 or 20 μ g/ml did not affect acetylcholine-induced AV block and norepinephrine-induced nodal rhythm.

Introduction

The effect of endotoxin on cardiac function has been discussed by many investigators (1, 4, 13). Kutner and Cohen (9) showed that endotoxin did not affect myocardial contractility of the cat papillary muscle preparation. More recently Hinshaw et al. (6) demonstrated that endotoxin at doses sufficient to induce shock did not influence cardiac function, left ventricular contractile force and oxygen uptake. We (2) also reported that concentrations of endotoxin equivalent to those producing shock when given intravenously did not affect the S-A pacemaker activity although larger amounts of endotoxin induced a slight negative chronotropic effect, using the in situ perfusion method of the sinus node artery of dogs. On the other hand, heart conductivity has not been investigated although it has an important role on heart function in case of cardiac arrhythmia. Siegel et al. (14) and Bell and Thal (16) have demonstrated myocardial failure in septic shock in patients. The present study was designed to investigate the effect of endotoxin on AV conductivity, using the direct in situ perfusion method of the AV node artery of dogs under constant flow rate (3, 12).

On the other hand, a single injection of endotoxin at doses of 100 to 300 μg did not affect AV conductivity in ECG recordings. The injection of 1 mg of endotoxin induced PR prolongation more than 30% in 2 out of 6 dogs. Higher plasma concentration above 20 $\mu\text{g}/\text{ml}$ of endotoxin occasionally induced a negative chronotropic effect and PR interval was gradually restored to its initial level within 5 minutes (Fig. 2).

In one case out of six, second degree AV block, 2:1 block, was observed. In one instance, a higher concentration of endotoxin (200 $\mu\text{g}/\text{ml}$) did not induce PR prolongation although 1 μg of acetylcholine easily caused an AV block. Atropine, 30 μg , blocked the effect of acetylcholine given into the AV node artery but did not prevent the AV block induced by endotoxin. The data is summarized in Table I.

Effect of endotoxin on acetylcholine and norepinephrine. Endotoxin did not modify the effect of acetylcholine as shown in table II.

Norepinephrine, 0.3 to 1 μg , induced AV nodal rhythm constantly when given into the AV node artery. This effect of norepinephrine, also, was not affected by treatment with a large amount of endotoxin administered into the AV node artery (Fig. 3). Response to norepinephrine was prevented by treatment with 10 μg of propranolol injected into the AV node arterial inflow.

Discussion

It is generally believed that the anatomical region with the greatest delay in the transmission of impulses is a narrow zone at the atrial margin of the AV node and this region has the lowest electrical threshold. It has been mentioned that AV block occurs in the fibers of the upper or atrial portion of the AV node, whether it is caused by acetylcholine, hypoxia, high stimulus rate or a combination of these factors (7). Lumb et al. (11) described that the posterior artery =

Methods

Six mongrel dogs weighing 10 to 15 kg were used. The animals were anesthetized with intravenous sodium pentobarbital, 30 mg/kg, and tracheotomized for the artificial respiration by a Harvard respirator. The chest was opened through the 5th right intercostal space and the heart was kept in its original position by making a pericardial cradle. Both vagi were cut at the midcervical level. The posterior septal artery, i.e., so-called "AV node artery" was cannulated and perfused with its blood introduced from the left carotid or the femoral artery under constant flow rate of 4 to 6 ml/min for keeping the correct plasma concentration of endotoxin. The preparation used for perfusion of the canine AV node artery was described in previous papers (3, 12). Systemic blood pressure in the femoral artery was continuously measured by a Grass Polygraph recorder. The degrees of AV block were recorded by use of lead II of ECG at the paper speed of 25 or 50 mm/sec. Sodium heparin, 500 U/kg, was given intravenously at the beginning of the perfusion and 200 U/kg was added at 1-hr intervals. The compounds used were acetylcholine chloride, atropine sulfate, dl-norepinephrine hydrochloride and dl-propranolol hydrochloride. These compounds were injected by use of a microinjector at a volume of 0.01 to 0.05 ml in a period of 4 seconds. The injection of 0.1 to 1 ml of saline or 5% glucose solution did not induce any remarkable dromotropic effect. Endotoxin (Difco, Escherichia coli) suspended in 5% glucose was given into the AV node artery by a microinjector, an injector of 1 ml or an infusion pump (Harvard Apparatus).

Results

Effect of acetylcholine and endotoxin injected into the AV node artery.

When a single dose of 1 to 10 μ g of acetylcholine was injected into the AV node artery, AV block was characteristically observed on ECG recordings as shown in figure 1.

AV node artery passed anteriorly to the left and inferior to the termination of the coronary sinus. It passed inferior to the AV node and closely followed the course of the bundle of His. In the present study, AV conduction block was readily elicited by 1 to 10 μ g of acetylcholine but no conduction disturbance was observed at a plasma concentration of 1 μ g/ml of endotoxin (inducing shock in the animal). Endotoxin at a plasma concentration of 10 μ g/ml occasionally induced PR prolongation. In a previous paper the effect of endotoxin on pacemaker activity of the S-A node was demonstrated, using the direct perfusion method of the canine sinus node artery (2). Endotoxin did not induce a chronotropic effect at a plasma concentration level inducing shock, and at higher doses it produced a slight negative chronotropic response. More recently, Hinshaw et al. showed that endotoxin has no detrimental effect on the isolated heart perfused by a donor dog (6).

On the other hand, Solis and Downing (15) and Kadowitz and Yord (8) reported that endotoxin influenced ventricular contractility when given intravenously. Jeffer et al. reported that the electrical and mechanical performance of papillary muscles isolated from cats in late postoligemic shock were significantly impaired (10). They and other investigators (5) hypothesized the presence of toxic factor in the plasma of animals in shock which depressed the heart. In the present study, endotoxin had no significant chronotropic effect in dogs except in high concentrations.

Endotoxin has been shown to increase the vascular reactivity to catecholamines by Zweifach et al. (17). More recently, Bhagat et al. reported that endotoxin pretreatment reduced the sensitivity to norepinephrine in the isolated atrial muscle of the guinea-pig (1). In this study, endotoxin treatment did not reduce the response to norepinephrine. So, it is suggested that endotoxin does not modify an action of norepinephrine directly, but it may indirectly affect the heart.

REFERENCES

1. Bhagat, B., Cavanagh, D., Merrild, B. N., Rana, M. W. and Rao, P. S., Brit. J. Pharmacol. 39:688, 1970.
2. Chiba, S. and Nakajima, T., Proc. Soc. Exp. Biol. 137:1429, 1971.
3. Chiba, S. and Hashimoto, K., Tohoku J. Exp. Med. 95:411, 1968.
4. Gilbert, R. P., Physiol. Rev. 40:245, 1960.
5. Gomez, O. A. and Hamilton, W. F., Circulation Res. 14:327, 1964.
6. Hinshaw, L. B., Archer, L. T., Miller, J. A., Greenfield, L. J., and Guenter, C. A., Physiologist 13:222, 1970.
7. Hoffman, B. F. and Cranefield, P. F., The Electrophysiology of the Heart, McGraw-Hill, New York, 1960.
8. Kadowitz, P. J. and Yard, A. C., Europ. J. Pharmacol. 9:311, 1970.
9. Kutner, F. R. and Cohen, J., J. Surg. Res. 6:83, 1966.
10. Lefer, A. M., Cowgill, R., Marshall, F. F., Hall, L. M. and Brand, E. D., Amer. J. Physiol. 213:492, 1967.
11. Lumb, G., Shacklett, R. S. and Dawkins, W. A., Amer. J. Pathol. 35:467, 1959.
12. Nadeau, R. A. and Amir-Jahed, A. K., Rev. Canad. Biol. 24:291, 1965.
13. Priano, L. L., Wilson, R. D. and Traber, D. L., Proc. Soc. Exp. Biol. 135:495, 1970.
14. Siegel, J. H., Greenspan, M. and Del Guercio, L. R., Ann. Surg. 165:504, 1967.
15. Solis, R. T. and Downing, S. E., Amer. J. Physiol. 211:307, 1966.
16. Bell, H. and Thal, A., Postgrad. Med. 48:106, 1970.
17. Zweifach, B. W., Nagler, A. L. and Thomas, L., J. Exp. Med. 104:881, 1956.

TABLE I
*Effect of Endotoxin and Acetylcholine on AV Conduction Induced
 by Injection into the AV Node Artery of Dogs "In Situ"*

Compound	No. of dogs	Heart rate (beats/min)	Freq. of PR-prolongation more than 30 per cent	Freq. of 11 degree AV block	Freq. of Complete AV block
A. Endotoxin i.a., 4 sec					
100 µg	6	147 ± 7	0/6	0/6	0/6
300 µg	6	148 ± 10	0/6	0/6	0/6
1 mg	6	151 ± 11	2/6	0/6	0/6
B. Endotoxin i.a. continuous infusion** (plasma conc.)					
20 µg/ml	6	145 ± 8	1/6	1/6	0/6
200 µg/ml	3	150 ± 2	2/3	1/3	0/3
C. Acetylcholine i.a. 4 sec					
1 µg	5	144 ± 11	5/5	2/5	1/5
3 µg	6	146 ± 8	6/6	6/6	4/6
10 µg	6	150 ± 12	6/6	6/6	6/6

Results are given as mean ± S.E. — * PR intervals are calculated from ECG recordings of lead II. — ** Endotoxin is infused for 5 to 10 min.

TABLE II

Effect of Endotoxin on Acetylcholine-Induced AV Block
and Norepinephrine-Induced AV Nodal Tachycardia

Drugs (μ g)	No. of dogs	Control heart rate (Sinus rhythm, beats min)	Effects of drugs
Acetylcholine			Second and complete AV block (sec)
1 μ g	5	144 \pm 5	Before endotoxin 4 \pm 0.4 After endotoxin (1 mg, or 20 μ g ml) 4 \pm 0.4
10 μ g	6	147 \pm 4	8 \pm 2.2 9 \pm 1.8
Norepinephrine			AV nodal rhythm
			Before endotoxin After endotoxin (1 to 3 mg or 20 μ g ml)
			Maximum rate (beats min)
			Duration (sec)
0.3 μ g	3	147 \pm 18	158 \pm 17 9 \pm 1.4
1.0 μ g	5	146 \pm 4	168 \pm 5 20 \pm 1.2 156 \pm 12 8 \pm 1.7 165 \pm 5 18 \pm 1.4

EXP. DOG 2.

ECG Lead II

1 sec

• ACh 1 μ g

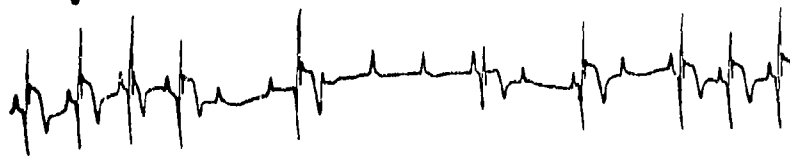


FIG. 1

Absence of effect of endotoxin on acetylcholine (ACh)-induced AV block. A plasma concentration of endotoxin is about 20 μ g/ml. ECG, lead II.

EXP. DOG 2.

0.5 sec

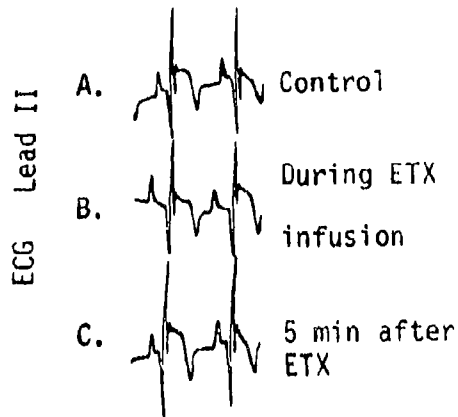


FIG. 2

PR prolongation induced by a continuous infusion of endotoxin (ETX) at a plasma concentration of 200 μ g/ml. ECG, lead II. A. Control. B. During endotoxin infusion. C. 5 min after endotoxin infusion.

EXP. DOG 1.

ECG Lead II

1 sec

• Norepinephrine 1 μ g

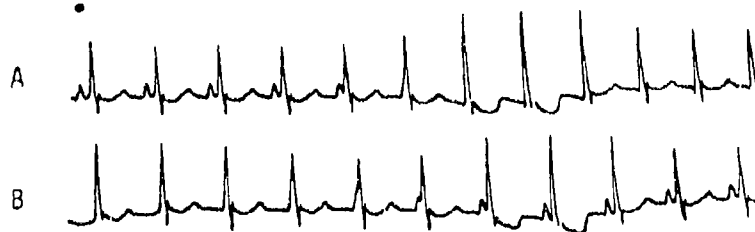


FIG. 3

Absence of blocking effect of endotoxin on norepinephrine-induced nodal rhythm. A plasma concentration of endotoxin is about 20 μ g/ml. ECG, lead II.