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Effects of perivascular nerve stimulation on the contraction and automaticity of the blood-perfused canine papillary muscle

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Summary

1. Effects of ventricular perivascular nerve stimulation (p.n.s.) on the ventricular contractility and idioventricular rate were investigated with the blood-perfused papillary muscle of the canine right ventricle.

2. Perivascular nerve stimulation of supramaximal voltage and 1 msec pulse-duration caused a definite positive inotropic response at a frequency of 1 Hz, which gradually reached a maximum at 15 to 20 Hz when the papillary muscle was electrically driven at 120 beats/min at a constant temperature of 38–39° C. The frequency–response curve was sigmoid.

3. The spontaneous regular idioventricular rate of 46 ± 4 beats/min was accelerated to at most 60 ± 4 beats/min ($n=11$) by p.n.s. The stimulus frequency–response relations between chronotropic and inotropic responses to p.n.s. were almost the same.

4. Tetrodotoxin blocked completely the responses to p.n.s. while it had little or no effect on the inotropic response to exogenous noradrenaline.

5. Positive inotropic responses to p.n.s. were diminished by β-adrenoceptor blocking agents (alprenolol, propranolol and pindolol) and were enhanced during infusion of cocaine.

6. In reserpine- or guanethidine-pretreated muscles, p.n.s. as well as field stimulation produced negative inotropic responses, which were enhanced by physostigmine and were blocked by atropine.

7. Hexamethonium enhanced slightly the positive inotropic responses to p.n.s. as well as field stimulation.

8. It was concluded that the perivascular nerves of the coronary artery of the canine ventricle are mainly composed of postganglionic adrenergic fibres but there are also pre- and postganglionic cholinergic nerve fibres.

Introduction

Though the important roles of autonomic nerves in the ventricular myocardium have been well documented in a number of preparations *in vivo* (DeGeest, Levy & Zieske, 1964; Szentivanyi, Pace, Wechsler & Randall, 1967; Vassalle, Levine & Stuckey, 1968; Priola & Fulton, 1969), and *in vitro* (Vincenzi & West, 1963; Blinks, 1966; Endoh & Hashimoto, 1970), field stimulation has been the only method used to elucidate direct effects of the autonomic nerves on ventricular myocardial contraction (Vincenzi & West, 1963; Blinks, 1966; Endoh & Hashimoto, 1970). Thus, effects of perivascular nerve stimulation on the per-

formance of the myocardium remain unknown. Recently we have successfully stimulated the coronary perivascular nerves to the ventricular myocardium of the blood-perfused canine papillary muscle preparation. In this paper we report the results of a pharmacological analysis of these effects using tetrodotoxin (TTX), cocaine, β-adrenoceptor blocking agents, reserpine, guanethidine, physostigmine, atropine and hexamethonium.

Methods

Forty-two mongrel dogs of either sex weighing 7 to 12 kg were anaesthetized with ether. After 200 u/kg of sodium heparin, i.v., the heart was excised. The anterior septal artery was carefully isolated about 1 cm from its origin in the left coronary artery. The septal artery was cannulated and the anterior papillary muscle was perfused at a constant pressure of 100 mmHg through the artery with arterial blood of a donor dog. The details of dissection, fixation of the papillary muscle and the experimental set-up have been described previously (Endoh & Hashimoto, 1970). The prepared muscle was kept at 38 to 39° C in a moist chamber. Donor animals were anaesthetized with sodium pentobarbitone, 30 mg/kg i.v. and the blood taken via a peristaltic pump from the carotid artery, circulated through the papillary muscle and returned to the femoral vein. Sodium heparin 300 u/kg, was given at the beginning of the perfusion and 100 u/kg was added at 1 h intervals. The muscle was stimulated via silver electrodes brought into contact with the root of the papillary muscle using 0·4 to 0·8 V which was approximately twice the threshold voltage, with 5 msec duration pulses at 2 Hz. No electrorelease of autonomic transmitters occurs with these conditions of stimulation. Strong field stimulation, however, with 10 V for 15–30 s produces the electrorelease of autonomic transmitters. The former condition is described as standard field stimulation and the latter one as test field stimulation in the present study. The perivascular nerve stimulation (p.n.s.) was provided by a Nihon Kohden MSE-3 electronic stimulator through additional silver electrodes placed around the septal artery 1·0 to 1·5 mm apart. The p.n.s. was usually applied for 15 s, using rectangular pulses of 1 ms duration and supramaximal voltage, normally 8 to 12 V, at frequencies of from 1 to 30 Hz. The muscle was stretched with a tension of 1 g during the equilibration period of about 1 h and this resting tension was kept constant. Isometric tension and the maximum rate of tension development (dT/dt) were recorded on an ink-writing rectigraph (San-ei Sokki Instrument) through a carrier preamplifier (Nihon Kohden RP-3), and RC circuit and a high gain amplifier (Nihon Kohden RDH-2). Ventricular automaticity was determined by a cardiotachogram which was triggered by dT/dt. The drugs used in these experiments were (−)-noradrenaline hydrochloride, acetylcholine chloride, atropine sulphate, physostigmine salicylate (Merck), tetrodotoxin (TTX, Central Laboratories of Sankyo Co.), (±)-alprenolol hydrochloride (kindly provided by Dr. B. Åblad, AB Hässle), (±)-propranolol hydrochloride (Sumitomo Chemicals), (±)-pindolol (kindly provided by Dr. K. Saameli, Sandoz), cocaine hydrochloride, guanethidine sulphate (Ciba), reserpine, hexamethonium bromide (C_6, Yamanouchi). Drugs were injected in a volume of 0·01 to 0·03 ml close to the septal artery in a period of 4 s using a micro-injector (Jintan Terumo Co.). Doses of drugs except tetrodotoxin, pindolol and reserpine are given in terms of the salts. Reserpine in a dose of 0·1 mg/kg was given 48 and 24 h before the experiments to eight animals according to procedures used by Blinks & Waud (1960).

TABLE 1. *Effects of perivascular nerve stimulation, noradrenaline and test field stimulation on the isometric tension and dT/dt of the canine papillary muscle. Resting tension standardized to 1 gramme. The nerve stimulation and field stimulation were applied for 15 seconds. Number of muscles in parentheses. The values given (mean ± S.E.) are calculated as percentage changes*

	Frequency of the nerve stimulation (Hz)						Noradrenaline	Test field stimulation
	1	2	3	5	8	10	0·03 μg	
Tension	5·6±0·8 (21)	15·8±2·1 (20)	23·2±2·9 (20)	40·7± 4·9 (21)	64·8± 6·8 (20)	73·4± 9·0 (21)	52·4±6·7 (17)	40·5± 6·9 (9)
d*T*/d*t*	11·2±1·6 (21)	29·0±4·3 (20)	47·1±6·7 (20)	89·4±11·9 (21)	148·6±21·6 (20)	178·4±27·0 (21)	105·1±13·4 (17)	71·8±13·2 (9)

TABLE 3. *Effects of pharmacological agents on the contractile responses to perivascular nerve stimulation, noradrenaline and test field stimulation. The values given (mean ± S.E.) are calculated as percentage changes*

	Frequency of nerve stimulation (Hz)									Noradrenaline	Test field stimulation
	1	2	3	5	8	10	15	20	30	0·03 μg	
Control	3·6±1·0 (5)	8·8±1·9 (4)	12·3±2·0 (4)	21·4±3·9 (5)	25·6±2·4 (4)	31·4±1·8 (5)	32·9 (2)	52·7 (2)		43·4±3·8 (4)	
Tetrodotoxin	0 (5)	0 (4)	0 (4)	0 (5)	0 (4)	0·6±0·6 (5)	0 (2)	2·4 (2)		36·1±14·4 (4)	
20 min after TTX	0 (2)	0 (2)	2·2 (2)	7·6 (2)	14·1 (2)	22·7±3·4 (5)	38·6 (1)	41·3 (2)			
Control	3·6±1·2 (6)	11·8±3·4 (7)	18·8±4·6 (8)	34·9±7·6 (8)	53·2±10·5 (8)	62·0±11·1 (9)	35·6 (2)	136·4 (2)		50·0±7·2 (5)	32·4 (2)
Alprenolol	0 (6)	3·4±1·4 (7)	5·6±1·4 (8)	11·8±3·6 (8)	20·3±4·2 (8)	31·8±8·8 (9)	10·1 (2)	72·7 (2)		23·1±9·0 (5)	7·8 (2)
Control	5·3±1·6 (6)	13·7±4·7 (6)	20·7±6·1 (6)	35·2±8·7 (6)	58·9±12·1 (6)	69·4±12·6 (6)	92·8±28·3 (3)	85·2±23·5 (4)	85·2±19·1 (5)	54·5±11·4 (5)	32·5±4·0 (5)
Guanethidine	0 (6)	−0·6±0·4 (6)	−1·3±0·7 (6)	−1·4±0·6 (6)	−2·4±1·2 (6)	−2·7±0·8 (6)	−2·8 (2)	−3·5±1·2 (4)	−4·4±1·7 (5)	75·0±12·0 (3)	−14·7±3·8 (5)
Control	5·6±2·6 (4)	12·9±4·9 (4)	23·7±7·8 (4)	45·1±12·1 (4)	69·0±16·3 (4)	72·0±11·5 (4)		56·6 (1)		34·5±4·3 (3)	30·8 (2)
Cocaine	8·6±4·3 (4)	21·5±7·3 (4)	32·8±9·2 (4)	59·9±14·1 (4)	90·0±18·6 (4)	101·0±15·8 (4)		224·8 (1)		59·7±9·8 (3)	42·6 (2)
Control						92·5±33·8 (6)					67·3±12·5 (6)
Hexamethonium						114·9±55·2 (6)					109·5±25·5 (6)

Results

Effects of p.n.s. on isometric tension and dT/dt of the papillary muscle paced at 2 Hz

The muscle was driven by standard field stimulation and the effect of number of pulses of p.n.s. on the contraction was examined. The duration of stimulation was kept constant at 15 s and the frequency of p.n.s. was changed from 1 to 2, 3, 5, 8 and 10 Hz: i.e., the number of pulses was increased from 15 to 30, 45, 75, 120 and 150 for each period of stimulation. Slight but significant positive inotropic effects and an acceleration of the dT/dt were elicited by p.n.s. which increased gradually as the frequency of p.n.s. was raised. Results are summarized in Table 1.

The acceleration of the dT/dt in response to p.n.s. occurred almost in parallel with the increase in isometric tension. Test field stimulation and exogenous noradrenaline (0·03 μg) also increased isometric tension and accelerated dT/dt in the same manner as did p.n.s. (Table 1). The response of the same preparation remained almost constant throughout an experiment lasting over four hours.

The number of pulses of p.n.s. was then kept constant at 150 in each period of stimulation and effect of the changes in the frequency of p.n.s. was tested in the next series of experiments. The frequency of p.n.s. used was 10, 15, 20 and 30 Hz. In consequence, the duration of stimulation was reduced from 15 s to 10, 7·5 and 5 seconds. Contractile responses to p.n.s. were almost equal at all frequencies. Results are summarized in Table 2. Responses to p.n.s. were not significantly different but appeared less at 10 Hz than at other frequencies. This may be caused by wash-out of the released transmitter by the blood flowing through the myocardium since the duration of p.n.s. was longer than at other frequencies. The effect of noradrenaline was also studied in each preparation (Table 2).

Effects of p.n.s. on the idioventricular rate

The idioventricular rate was 42 ± 3 beats/min (mean $\pm$ S.E.) in 18 normal papillary muscles and was 43 ± 3 beats/min in four reserpine pretreated muscles. The rate was regular with a few extrasystolic contractions and changed little throughout the experiments. An acceleration of the idioventricular rate accompanied by increases in isometric tension and dT/dt was induced by p.n.s. as shown in Figure 1.

The rate was accelerated slightly even at 2 Hz, more markedly at 3 and 5 Hz, and reached a maximum at 10 to 20 Hz as shown in Figure 1. The control rate of 46 ± 4 beats/min was accelerated to 60 ± 4 beats/min ($n=11$) by the maximum p.n.s. (10 to 30 Hz for 30 s). A dose of 0·03 μg noradrenaline accelerated the control rate of 43 ± 3 beats/min to 56 ± 4 beats/min ($n=10$). Noradrenaline in a

TABLE 2. *Effects of perivascular nerve stimulation and noradrenaline on the isometric tension and dT/dt of the canine papillary muscle. The number of the pulses of nerve stimulation was kept constant at 150 and the frequency of electrical stimulation was changed. Numbers of muscles in parentheses. The values (mean ± S.E.) given are calculated as percentage changes.*

	Frequency of nerve stimulation (Hz)				Noradrenaline
	10	15	20	30	0·03 μg
Tension	74·5±9·1 (7)	97·2±14·5 (7)	95·6±15·8 (7)	89·9±13·8 (7)	57·3±7·2 (7)
dT/dt	116·7±16·5 (7)	157·6±22·6 (7)	148·4±25·0 (7)	148·9±24·0 (7)	106·4±11·3 (7)

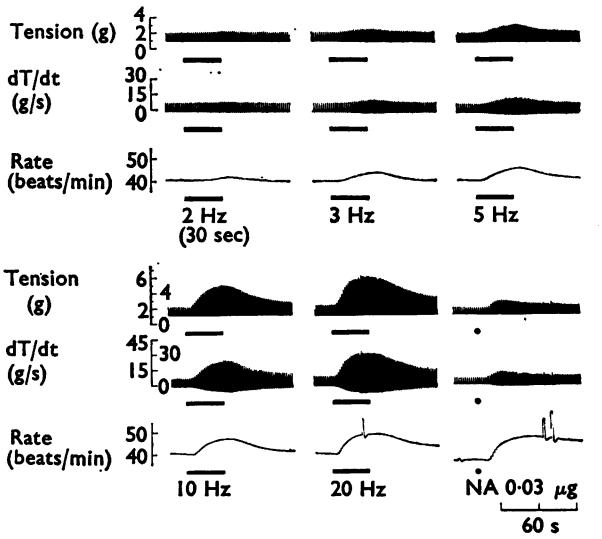

FIG. 1 Effects of perivascular nerve stimulation on the isometric tension, dT/dt and the idioventricular rate of the blood-perfused canine papillary muscle contracting spontaneously. Stimulation was by pulses of 1 msec, 8 V at various frequencies for 30 s. Length of black bars and numbers below each record represent the duration and the frequency of the nerve stimulation. NA, noradrenaline.

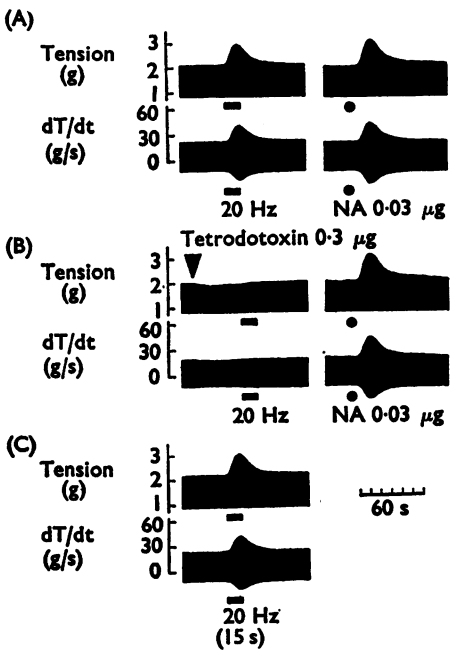

FIG. 2. Blocking effect of 0·3 μg of TTX on the contractile responses to perivascular nerve stimulation (p.n.s.) and the recovery of the responses to p.n.s. from the blocking effect of TTX. The papillary muscle was driven at 2 Hz. The p.n.s. was by pulses of 1 msec, 12 V at 20 Hz. (A), control responses to p.n.s. and noradrenaline (NA); (B), responses 60 and 150 sec after the administration of 0·3 μg of TTX; (C), responses to p.n.s. 20 min after TTX.

TABLE 4. *Effects of pharmacological agents on changes of dT/dt of the papillary muscle in response to perivascular nerve stimulation, noradrenaline and test field stimulation. The values given (mean±S.E.) are calculated as percentage changes*

	Frequency of nerve stimulation (Hz)									Noradrenaline	Test field stimulation
	1	2	3	5	8	10	15	20	30	0·03 μg	
Control	8·3±2·4 (5)	21·1±5·0 (4)	33·7±3·8 (4)	70·4±15·6 (5)	109·5±8·6 (4)	159·8±8·6 (5)	130·7 (2)	207·4 (2)		148·6±11·7 (4)	
Tetrodotoxin	0 (5)	0 (4)	0 (4)	0 (5)	0 (4)	0 (5)	0 (2)	2·8 (2)		140·7±50·3 (4)	
20 min after TTX	0 (2)	0 (2)	24·3 (2)	53·1 (2)	102·5 (2)	128·1±23·9 (5)	157·1 (1)	166·3 (2)			
Control	8·9±3·0 (6)	26·2±7·5 (7)	43·5±11·8 (8)	84·1±21·8 (8)	145·9±37·7 (8)	182·0±40·0 (9)	77·6 (2)	153·5 (2)		83·0±13·2 (5)	
Alprenolol	0 (6)	9·6±3·8 (7)	13·9±3·8 (8)	33·0±10·0 (8)	67·7±21·7 (8)	95·7±31·0 (9)	20·5 (2)	69·1 (2)		50·8±13·8 (5)	
Control	10·2±3·3 (6)	27·0±10·3 (6)	38·4±12·0 (6)	64·3±18·4 (6)	113·3±35·9 (6)	123·2±33·4 (6)	138·7±39·6 (3)	136·5±38·4 (4)	140·8±35·9 (5)	100·1±21·7 (5)	60·6±15·1 (5)
Guanethidine	0 (6)	−1·6±1·0 (6)	−4·7±2·5 (6)	−4·4±2·1 (6)	−6·9±3·2 (6)	−9·0±3·5 (6)	−3·9 (2)	7·3±3·0 (4)	−8·5±0·7 (5)	221·0±64·5 (3)	−27·6±3·3 (5)
Control	7·2±3·0 (4)	18·4±6·8 (4)	34·5±10·9 (4)	65·6±19·3 (4)	112·5±34·1 (4)	119·1±33·6 (4)		64·4 (1)		51·2±10·5 (3)	28·1 (2)
Cocaine	14·3±6·0 (4)	36·1±12·0 (4)	53·4±18·6 (4)	100·9±32·5 (4)	175·2±58·8 (4)	198·0±59·0 (4)		206·3 (1)		99·7±15·1 (3)	64·7 (2)
Control						114·3±56·2 (6)					80·3±9·2 (6)
Hexamethonium						146·4±74·5 (6)					134·9±23·5 (6)

dose of 0·1 μg usually caused a few extrasystolic contractions and the idioventricular rate could not be determined. The acceleration of the idioventricular rate by p.n.s. was less prominent than the increase of contractile force, while the reverse was the case following the use of noradrenaline. The idioventricular rate during p.n.s. rarely exceeded 70 beats/min.

Effects of tetrodotoxin

Effect of tetrodotoxin on the inotropic response to p.n.s. was examined by using a single dose of 0·3 or 1 μg, since in a previous paper it was demonstrated that these doses of tetrodotoxin were sufficient to abolish only the neurally mediated positive inotropic responses induced by field stimulation (Endoh & Hashimoto, 1970). These doses of tetrodotoxin blocked completely the contractile response to p.n.s. 1 min after close-arterial injection, while the inotropic response to noradrenaline was not influenced. The control response to p.n.s. recovered in 20 to 30 min after the administration of 0·3 μg of tetrodotoxin as shown in Figure 2. The blocking effect of tetrodotoxin on the changes in isometric tension and dT/dt in response to p.n.s. at various frequencies was investigated in nine papillary muscles and the results are given in Tables 3 and 4.

Effects of β-adrenoceptor blocking agents

Alprenolol in a dose of 3 μg reduced the positive intropic responses to p.n.s., noradrenaline and test field stimulation to almost the same extent. Recovery to control values took 30 to 50 minutes. These results from nine muscles are presented in Tables 3 and 4. Propranolol in a dose of 3 μg decreased the responses to p.n.s. and noradrenaline in a similar manner to alprenolol. Recovery was complete 30 to 50 min after administration. Pindolol, the most potent β-adrenoceptor blocking agent used in these experiments (Hashimoto, Endoh, Tamura & Taira, 1970), in a dose of 1 μg decreased markedly the positive inotropic responses to p.n.s. and the effect lasted over 90 minutes.

Effects of cocaine

The effect of cocaine infusion at 3 μg/min on the responses to p.n.s. noradrenaline and test field stimulation was examined. Typical results are illustrated in Fig. 3. Cocaine increased isometric tension and the dT/dt of contraction caused by p.n.s., noradrenaline and test field stimulation (Fig. 3A). The duration of the positive inotropic responses was also prolonged (Fig. 3B). The average effects of cocaine in four muscles are given in Tables 3 and 4.

Effects of guanethidine

Guanethidine in a dose of 50 μg produced a pronounced positive inotropic effect as reported in a previous paper (Endoh & Hashimoto, 1970). The positive inotropic response to p.n.s. was converted to a negative one after five successive administrations of 50 μg of guanethidine, while the response to noradrenaline remained unchanged (Tables 3 and 4).

After a total of 150 μg of guanethidine the response to test field stimulation was converted to a negative one, but p.n.s. at higher frequencies still produced a

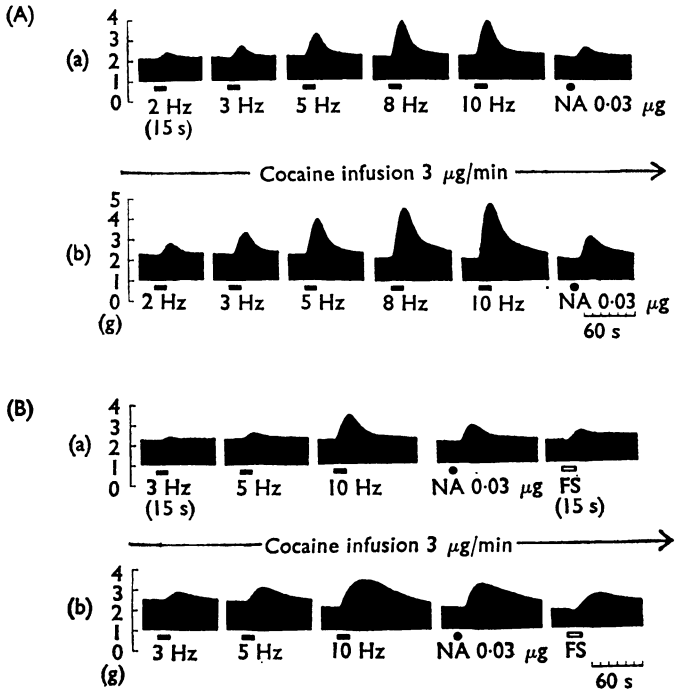

FIG. 3. Potentiating effects of cocaine on the contractile responses of the papillary muscle to perivascular nerve stimulation (p.n.s.) at various frequencies, noradrenaline (NA) and test field stimulation (FS). Cocaine was continuously infused via the cannula inserted in the septal artery at a rate of 3 μg/min. (A), An experiment in which cocaine potentiated mainly the contractile height; (B) an experiment in which cocaine increased the duration of the positive inotropic responses to p.n.s. (■), noradrenaline (●) and test field stimulation (□). (a), Control contractile responses; (b), contractile responses during cocaine infusion.

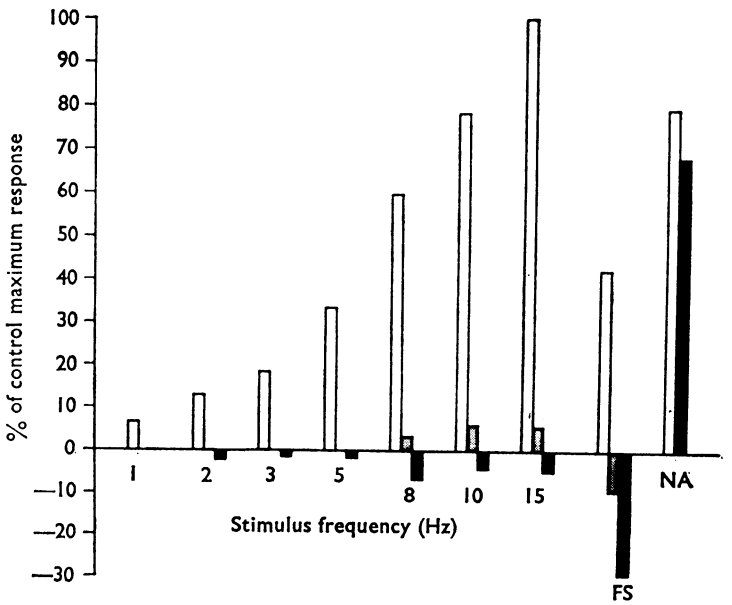

FIG. 4. Effects of guanethidine on the contractile responses to perivascular nerve stimulation (p.n.s.) at various frequencies, test field stimulation and 0·03 μg of noradrenaline (NA). □, Control percentage changes of the isometric tension; ▒, changes after 150 μg of guanethidine; ■, changes after 250 μg of guanethidine. The p.n.s. was by pulses of 1 ms-duration, 8 V at various frequencies of from 1 to 15 Hz for 15 seconds. Test field stimulation was by pulses of 5 ms, 10 V for 15 seconds.

positive response as shown in Fig. 4. After 250 μg of guanethidine, the responses to p.n.s. became negative, and test field stimulation caused a more pronounced negative inotropic response while the positive inotropic response to noradrenaline remained unchanged (Fig. 4).

Effects of reserpine

Papillary muscles removed from reserpine-pretreated animals were perfused with the blood from reserpine-pretreated donor dogs, and the effects of p.n.s., test field stimulation and tyramine were investigated in 4 preparations. While tyramine produced a long-lasting positive inotropic response in doses of from 0·3 to 30 μg in normal muscles as shown in Table 5, in reserpine-pretreated muscles tyramine produced only a small positive inotropic effect.

A slight but detectable negative inotropic response was observed during p.n.s. in three out of four reserpine-pretreated muscles at 10 to 30 Hz as summarized in

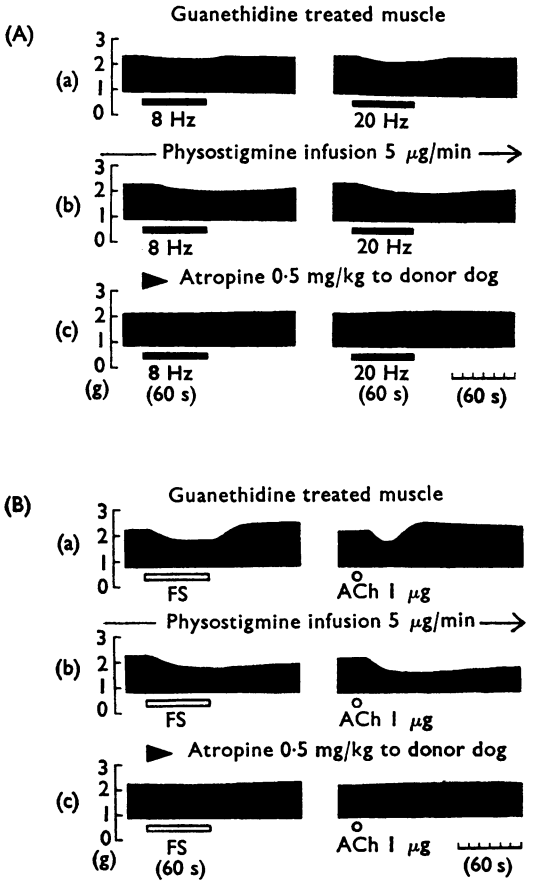

FIG. 5. Effects of physostigmine and atropine on the negative inotropic responses to perivascular nerve stimulation (p.n.s.) (A), and test field stimulation and 1 μg of ACh (B) in the guanethidine pretreated papillary muscle. (A) and (B) were responses in the same preparation. (a), Control contractile responses to p.n.s. (■), test field stimulation (FS, □) and ACh (○) in the guanethidine-treated muscle; (b), responses during the continuous infusion of physostigmine at a rate of 5 μg/min to the septal artery; (c), responses after the i.v. administration of 0·5 mg/kg of atropine to the donor dog.

TABLE 5. *Effects of tyramine and noradrenaline on the isometric tension and dT/dt of the papillary muscle. Numbers of muscles in parentheses. The values given (mean±S.E.) are calculated as percentage changes*

	Tyramine (μg)						Noradrenaline (μg)
	0·1	0·3	1	3	10	30	0·03
Tension	0·7±0·7 (2)	4·2±2·3 (3)	10·7±6·5 (7)	36·9±8·7 (7)	69·7±18·0 (7)	74·8±15·3 (4)	71·5±12·9 (4)
d*T*/d*t*	4·2±4·2 (2)	8·7±4·4 (3)	27·5±17·1 (7)	98·0±32·9 (7)	200·1±52·7 (7)	297·1±95·0 (4)	173·2±40·0 (4)

TABLE 6. *Effects of reserpine pretreatment on the contractile responses of the papillary muscle to perivascular nerve stimulation, field stimulation, tyramine, noradrenaline and acetylcholine. The values given (mean±S.E.) are calculated as percentage changes*

		Frequency of nerve stimulation (Hz)			Test field stimulation	Tyramine	Noradrenaline	ACh
		10	20	30		30 μg	0·03 μg	1 μg
Tension	Reserpine	−0·6±2·5 (4)	0·6±4·6 (4)	2·3±5·8 (4)	−17·6±0·8 (4)	8·9±5·7 (4)	36·8±2·8 (4)	−14·1±3·4 (4)
	Reserpine+ Guanethidine	−5·2 (2)	−6·0 (2)	−7·8 (2)	−22·9±1·9 (3)			
d*T*/d*t*	Reserpine	0±5·7 (4)	1·2±5·4 (4)	4·0±12·2 (4)	−11·5±3·4 (4)	15·2 ±11·7 (4)	136·1±19·8 (4)	−27·0±4·4 (4)
	Reserpine+ Guanethidine	−16·3 (2)	−17·4 (2)	−19·8 (2)	−18·6±3·4 (3)			

Table 6. Guanethidine treatment enhanced the negative inotropic effect caused by p.n.s. and test field stimulation as shown in Table 6. This indicates that reserpine pretreatment in these experiments was not sufficient for complete depletion of myocardial noradrenaline. The negative inotropic effect of acetylcholine was also investigated in these muscles to compare with the negative inotropic effects of p.n.s. and test field stimulation.

Effects of physostigmine and atropine

Effects of physostigmine and atropine on the negative inotropic responses to p.n.s., test field stimulation and acetylcholine were observed in six guanethidine treated muscles.

Physostigmine infusion in a dose of 5 μg/min enhanced the negative inotropic responses to p.n.s. as shown in Fig. 5A. The responses to test field stimulation and acetylcholine, 1 μg, were also enhanced in their duration in the same preparation as shown in Fig. 5B. Negative inotropic responses to p.n.s., test field stimulation and acetylcholine were completely blocked after i.v. administration of 0·5 mg/kg of atropine to the donor dog as shown in Figs. 5A and 5B. Physostigmine enhanced mainly the duration of response, while the maximum negative inotropic response to p.n.s. remained almost the same.

Effects of hexamethonium

The effects of hexamethonium on the contractile responses to p.n.s. and test field stimulation were investigated in six muscles. Hexamethonium in a dose of 300 μg blocked completely the nicotinic positive inotropic effect of acetylcholine with little or no effect on the positive inotropic response to noradrenaline (Endoh, Tamura & Hashimoto, 1970). The positive inotropic responses to p.n.s. were slightly enhanced after 300 μg of hexamethonium as shown in Tables 3 and 4, but this was not statistically significant. Enhancing effects of hexamethonium on the positive inotropic responses were significantly larger following test field stimulation (Tables 3 and 4).

Negative inotropic effects of p.n.s. and test field stimulation in the guanethidine-treated muscles were reduced by 300 μg of hexamethonium but were not completely blocked.

Discussion

The distribution of the autonomic nerves to the mammalian ventricular myocardium has been the subject of a number of recent studies; Priola & Fulton (1969) examined the distribution of vagosympathetic nerves to the four cardiac chambers using the paced isovolumic canine atria and ventricles; Szentivanyi *et al.* (1967) demonstrated the distribution of sympathetic nerve fibres in restricted areas of individual cardiac chambers. Vincenzi & West (1963), Blinks (1966) and Endoh & Hashimoto (1970) studied 'electrorelease', i.e., the release of autonomic transmitters by direct strong electrical stimulation of the isolated cardiac muscle (field stimulation). No description has been found of the effect of coronary perivascular nerve stimulation on the mechanical performance of the ventricular muscle. In the present study the inotropic effect of p.n.s. was investigated in the papillary muscles paced at 120 beats/minute. The positive inotropic response to

p.n.s. was dependent on the frequency of stimulation and was completely blocked by TTX, markedly decreased by β-adrenoceptor blocking agents (alprenolol, propranolol and pindolol) and potentiated by cocaine. These pharmacological effects indicate that the perivascular nerves of the ventricular coronary artery were mainly composed of adrenergic nerve fibres.

Although the isolated cat papillary muscle has been reported to lose its automaticity in physiological saline solution (Garb & Chenoweth, 1949), the blood perfused canine papillary muscle does not lose the regular automaticity of about 40 beats/min, which is not modified by reserpine pretreatment. Therefore it is unlikely that catecholamines in the circulating blood play a role in the maintenance of automaticity. Vassalle, Levine & Stuckey (1968) found that the idioventricular rate in dogs observed in the atrioventricular block produced by ligating the bundle of His was about 40 beats/min, which accords with the idioventricular rate obtained in the blood-perfused papillary muscle preparation. Since the acceleration of the idioventricular rate by p.n.s. in the present study was also quite similar to that obtained by stellate ganglion stimulation (Vassalle *et al.*, 1968), it is probable that the idioventricular rate in the present study is determined by Purkinje fibres and that adrenergic nerve fibres from the stellate ganglion control directly the automaticity of Purkinje fibres through the perivascular nerves. Relations of positive inotropic and chronotropic effects to frequencies of p.n.s. and the time courses of their onset and development (Fig. 1) accord well with the effects of stimulation of the stellate ganglion (Vassalle *et al.*, 1968) and cardiac nerves (Szentivanyi *et al.*, 1967) in the heart *in vivo*. These similarities may provide functional evidence that adrenergic nerve fibres reach the ventricular myocardium with the coronary vessels.

Since the demonstration of cholinergic innervation in the mammalian ventricular myocardium by DeGeest *et al.* (1964) their findings have been confirmed in various preparations. Stronger field stimulation of the blood perfused papillary muscle preparation produced a marked negative inotropic response in the guanethidine-treated muscles, and this was blocked by atropine and TTX (Endoh & Hashimoto, 1970). In this study, p.n.s. also produced a negative inotropic response in the reserpine- and guanethidine-treated muscles, which was enhanced by physostigmine and blocked by atropine. Thus cholinergic nerve fibres probably innervate the canine papillary muscle partly through the perivascular nerve plexus. However, there is a quantitative difference in response to the guanethidine treatment when tested by field stimulation and p.n.s. Field stimulation strong enough to cause release of the chemical transmitter readily produced a negative inotropic response after 150 μg of guanethidine. At the same time p.n.s. still caused a positive, though weak, inotropic response, and further addition of 100 μg of guanethidine was necessary for the induction of a negative inotropic response. Field stimulation produced a negative inotropic response before induction of the dominant positive response even in non-treated muscles, while p.n.s. caused only a positive inotropic effect. This shows clearly the adrenergic dominance following p.n.s.

In the present study, hexamethonium in a dose sufficient to block the nicotinic positive inotropic effect of ACh (Endoh *et al.*, 1970), enhanced slightly the positive inotropic response to p.n.s., though not significantly. The enhancement of the positive inotropic response to stronger field stimulation after hexamethonium was greater and significant. The differences between field stimulation and p.n.s. after

hexamethonium suggest also that cholinergic nerve fibres mainly innervate the ventricle directly rather than through the perivascular plexus. The positive inotropic responses to stronger field stimulation were enhanced more markedly after atropine than after hexamethonium (Endoh & Hashimoto, 1970). Thus we suggest that the cholinergic nerve fibres in the ventricular myocardium are partly preganglionic and partly postganglionic. Adrenergic nerve fibres in the ventricle are probably postganglionic and so not affected by hexamethonium.

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